A study of the holly leaf miner and its parasites

Woodhouse, C.

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by

G. Woodhouse B.Sc. (Hons.) 1977

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Abstract.

This short term research was conscerned with an Agromyzid parasite -Phytomyza ilicis,Curt, which attacks young unthickened leaves of Ilex aquifolium, causing the formation of brown- yellow blotches or mines on the leaf surface. Few papers related to P. ilicis have been published, and no recorded work has been given for the North East area of England, despite the fact that the holly leaf miner /holly tree association provides a useful system for the study of plant-insect relationships.

Aims of the investigation were to determine whether differences in levels of infestation existed between trees of varying age and sex within the same area. Variation in infestation with height and aspect was investigated, together with egg mortality. Later mortality factors by bird-attack and different species of parasites was also examined. The life-cycle of P.ilicis and its parasites was recorded from the mature larval- adult stages. Adult Sphegigaster flavicornis were recorded from suction-traps only.

The study was carried out within Hollingside Wood,Durham city G.R. NZ.276 405.Sixteen holly bushes were examined on the basis of their isolation or proximaty to other holly bushes, position with respect to other vegetation, height and diameter, degree of healthiness based on the number of leaves per twig, sex and position with repect to gradient within the main wood. Six suction-traps were set up within a group of three adjacent trees at varying height intervals within the University field-station. The size of bushes examined varied between 1.7-9.6 metres. Problems with sampling are discussed, and it was decided to sample between 325-4300 leaves depending on the size of the tree. The ideal sample size was 800-1000 leaves. Leaves were examined for the presence of eggs, the number of eggs per leaf, the position along the midrib, the number of mines per leaf and their position on the leaf. Each mine was examined for bird-attack, and for the presence of both larval and pupal parasites. Leaf-sections were obtained from each tree for leaves of the 1977 season, to determine whether cuticle thickness influenced the level of infestation between trees. Cuticle thickness was found to be unimportant in determining whether a leaf was mined or not, however cuticle thickness ultimately is important since adult P.ilicis can only attack young leaves where the cuticle is undeveloped.

A variation in the time of emergence for adult P. ilicis was observed when compared with accounts given by Miall and Taylor (1907), Downes(1931), although the general pattern of the life-cycle was similar.
No significant difference in the level of infestation was observed between trees, although total mine and egg number varied significantly. Spatial distribution did not appear to influence the density of mines, and no significant difference in population density with regard to position within Hollingside Wood was found. Aspect and height influenced both total mine and egg number.

Egg mortality was observed to vary between trees but differences were not significant.Egg density was observed to influence the viability of eggs within the leaf, but the relationship was density-independant.

Major sources of mortality were attack by feeding birds, attack

by the larval parasite, C. gemma, and by an undetermined mortality factor influencing survival in the early stages of development. Thepupal parasites observed were C.syma and S.flavicornis.

Contents.

1. Introduction

1a. The host, Ilex aquifolium

The holly tree Ilex aquifolium is widespread as an understorey component of many types of woodland; in scrub communities and as a hedgerow tree, often being planted for this reason. Its distribution is probably determined mainly by its sensitivity to prolonged frost. Peterkin and LLoyd (1967), found it to be absent from areas where the mean temperature of the coldest months of the year falls below - 0.5°C and it has been suggested that the eastern boundary of holly is determined by the degree of winter cold, (Iversen 1944).

De Candolle (1855) and Iversen (1944) also found holly distribution to be influenced by the mean temperature of the warmest month, being absent from areas where this did not exceed 12 °C. In Britain holly is naturally absent from areas where the July temperature fails to exceed 12.8 °C.
(Fig. 1)

In accordance with Peterkin and LLoyd (1967), no subspecies have been described, although 140 forms have been named, many of which are of horticultural origin (Dallimore 1908). Varieties have been based on the pendulous habit, colour of bark, yellow berries, variegated foilage, leaf shape and size, leaf curvature, number of spines and their abnormal occurance on the leaf surface (Elwes and Henry 1913).

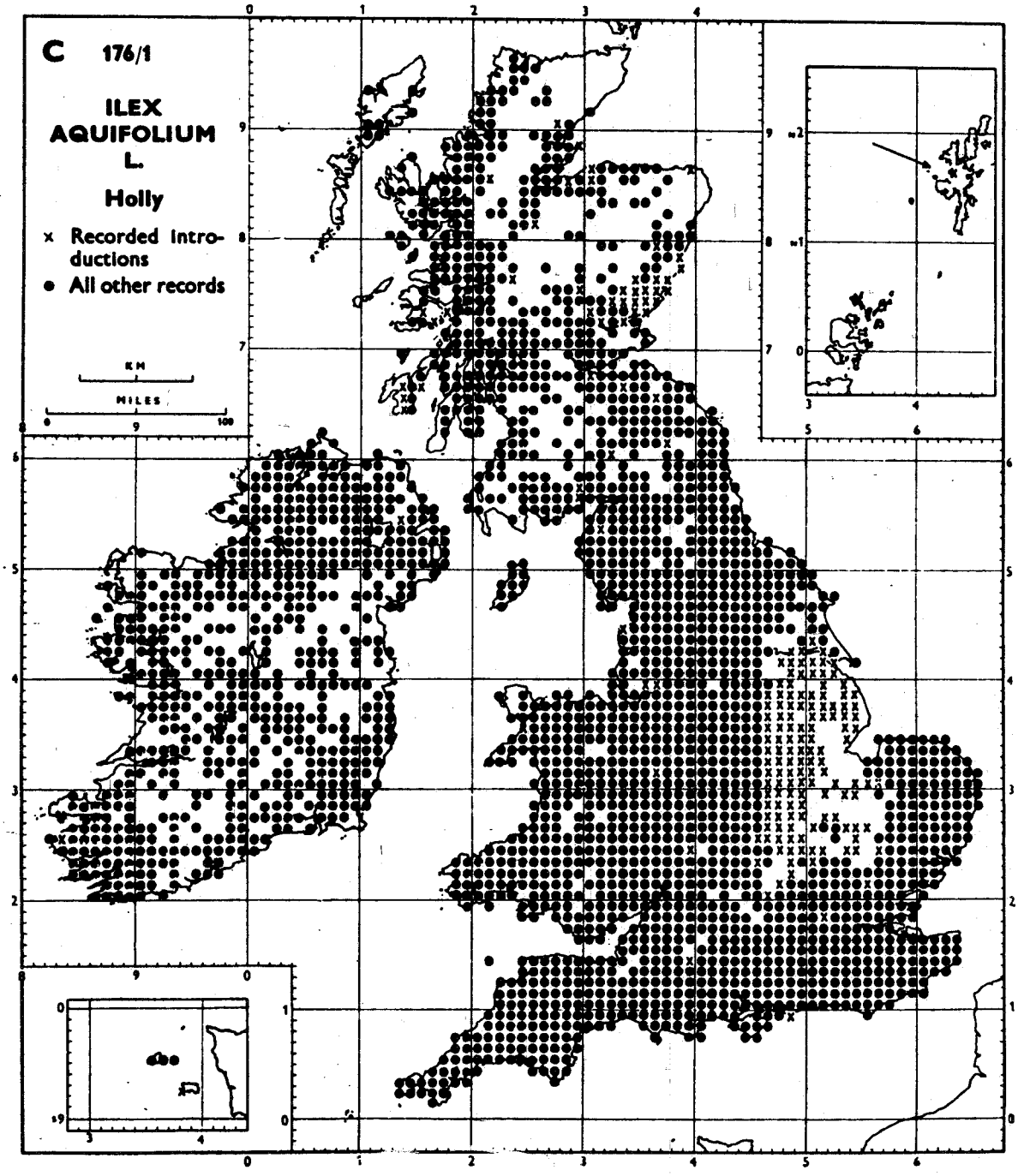
The leaves grow up to 10 cms long and 40 cm^2 in area, and are alternate and simple. Holly leaves increase in area in the second and subsequent years, although shaded leaves are thinner and larger in area. Their shape is ovate, elliptic or oblong, the margin being undulate or sinuate - dentate, with large spine pointed teeth. However leaves of older trees and individuals in shaded habitats tend to become entire, retaining only the sharp apical spine. The adaxial surface of the leaves is dark-green and glossy, the lower surface being yellow-green and non-glossy.

Shoot extension, leaf formation and root elongation commences early in May, and extends over 2 months, however in the Durham city area G.R. NZ 276 405, where sampling was carried out, climatic factors were less favorable, hence maximum leaf extension was retarded until late June.

1b. Insect species associated with Ilex aquifolium

Holly has a small number of associated insect species compared with other temperature region trees. Southwood (1961) hypothesized that the number of insect species associated with a tree was a reflection of the cumulative abundance of that tree in a particular country throughout recent geological history. Thus the dominant native trees should have most insect species, the recently introduced ones fewest.

Fig 1. The distribution of *Ilex aquifolium* in the British Isles.



Holly is reported to have 7 associated insect species in Britain, while that of oak (Quercus sp.), has 284, (Southwood 1961). The lack of insects on holly throughout the geographical range is marked and is assumed to be associated with either structural or biochemical features. Histological barriers to pathogenic and insect attack include the cuticle and epidermis (Ripley and Van Heerdan 1939; Tanton 1962; Agarwal 1969; Feeny 1970). Plants also contain secondary substances, i.e. phenols, tannins, lignins, alkaloides and glycosides which reduce infection in specific species of plants and digestion by insect feeders. (Fraenkel 1953, 59, 69; Lipke and Fraenkel 1956, Wood 1967).

Feeny (1970), found that the concentration in the spring of feeding caterpillers of the winter moth Operophtera brumata L., and other species of Lepidoptera on oak trees in England, were related to seasonal changes in the texture and chemical composition of the leaves. Leaf toughness was though to be the chief proximate factor preventing winter moth larvae from feeding normally on mature oak leaves, the most important ultimate factor being seasonal decline in available nitrogen. This decline is due to the decrease in leaf protein concentration and to increased concentration of leaf tannins. The ability of tannins to form complexes with proteins, and therefore reduce the availability of nitrogen to plant feeders, is believed to enhance their defensive function in plants. Thus although there appears to be a sufficient supply of food, it may not be available as suitable food for phytophagous insects and other herbivores. The fact that tannins are widespread within the plant kingdom may suggest a similar phenomenon exists with other plant/tree species.

Bracken, an understorey shrub, is also known to have a range of specific chemical defences. These include bound cyanide molecules in addition to ecdysones, the latter acting as a defence against insects since it prevents larvae from completing their life-cycle.

Other factors besides chemical defense may influence herbivore diversity associated with a particular plant species, i.e. it is known that the number of insects feeding on a plant is a function of the geographical range of the species and of the plant architecture. Thus trees being more complex than grasses, tend to have more available niches, resulting in reduced competition for food and space between insect feeders.

Few papers related to Ilex aquifolium and its parasites have been published, thus it is difficult to postulate reasons why insect feeders occur with such low frequency; although structural defence mechanisms appear most obvious.

Peterkin and LLoyd (1967) have summarized the insect feeders and plant pathogens associated with Ilex aquifolium as follows :-

Hemiptera	-	Aphididae	Aphis ilicis Kalt.
Lepidoptera	-	Geometridae	Acasis viretata Hb.
		Lycaeridae	Celastrina argiolus L. 1st brood mainly on flower buds of holly, second mainly on flower buds of ivy.

	Torticidae	Rhopobota naevana Hb.
Diptera -	Agromyzidae	Phytomyza ilicis Curt.

Plant Pathogens (Moore 1959)

Armillaria mellea (Vahl ex. fr.) Krummer.
Helicobasidium purpureum Pat.
Phyllosticta aquifolina Grove (Peace 1962).
Vialaea insculpta (fr.) Saccardo.
Ceuthospora phacidiodes Grey.
Phytophora ilicis Buddenhagen and Young (Herridge 1963).

The most important parasite of Ilex aquifolium is the holly leaf miner, Phytomyza ilicis Curt, which spends the whole of the larval period within the leaf of the host plant, and is responsible for the formation of yellow-bronze blotches observed on the leaves of infected trees. The parasite is unusual amongst the Agromyzidae in that it pupates within the leaf. Other species of Agromyzidae i.e. Phytomyza conii and P. spondylii, on completion of feeding, leave the mine through a semicircular exit slit before pupation. (Hering 1951).

Few papers related to P. ilicis have been published since Miall and Taylor published their paper in 1907, on the structure and life history of the holly-fly. A small account is given in Lewis and Taylor (1974), but the main papers are an account by Cameron (1939) on the holly leaf miner and its parasites, and that by Owen (1975), related to the efficiency of blue-tits Parus caerulens, preying on the larvae of P. ilicis.

The reasons why so little work has centred around Ilex aquifolium and its insect parasites is probably related to its lack of economic importance in this country. Cameron's work in North America was initiated because of the need to control the unlimited spread of the accidentally introduced leaf miner to that country. On the west coast of British Columbia, European holly is extensively cultivated and since it cannot be successfully grown in other parts of North America, an industry has developed. However the holly leaf miner, unrestrained due to the absence of natural enemies in this area, has resulted in as many as 75 - 80 % of leaves being disfigured as a result of mine formation. Hence from a commercial point of view the trees are reduced in value and are also rendered less ornamental and attractive. The overall effect has not been demostrated, but it is possible that leaf-blotching may have adverse effects on the health of trees, due to the wholesale removal of photosynthetically active cells.

In Britain holly grows extensively, hence its economic importance is reduced.

The holly leaf miner/holly tree association provides a useful system for the study of plant/insect relationship. This relationship may be in the form of shelter, food or transport, and for P. ilicis is essential for the completion of the life-cycle.

Phytomyza ilicis provides a useful tool in the study of insect population dynamics. From mine examinations, mortality factors, parasite species attacking both larvae and pupae, and the number of healthy adults emerging can be determined and can be used in the construction of life-tables. Many variables contribute to mortality in a population, however the main fluctuations are usually due to only a few factors, i.e. key-factors. From analysis of life-tables it is possible to determine the key-factor for population change and density - dependant effects. (Morris 1959, Varley and Gradwell 1963). An example of the use of life-table data in determining key-factors is given by Williamson (1972), for the pine-looper moth, Bupalins piniarius, where the key-factor was larval mortality. However for this investigation key-factor analysis was impossible since P. ilicis was at the larval - pupal stage before the study was initiated, hence there was insufficient data.

2.1 Aims of the investigation.

The main aims of the investigation were to determine the life-cycle and population dynamics of Phytomyza ilicis in the Durham city area, since the only other work involved with parasites of the leaf miner was that carried out by Cameron (1939), working in Hampshire, Gloustershire, Buckinghamshire and Surrey during 1937 - 1938; no work having been reported for North East England.

Other aims were to determine differences in infestation levels between trees of varying age and sex within the same area. Levels of infestation were recorded for trees in close proximity and solitary trees. Possible variation in infestation with height and aspect was investigated. The numbers of eggs laid and mines formed was examined, (i.e. mortality in the early stages of development) to determine if the relationship is density - dependant for the trees examined, or whether a fixed proportion of eggs laid, fail to survive to produce visible mines.

Possible reasons for any differences in the number of mines laid per 100 leaves for each tree examined were investigated within the limits of a short term study. Factors which were thought to influence infestation were associated with leaf structure, nutrition levels within the leaves (Kennedy 1951)and the level of defence compounds i.e. tannins and phenols. The first of these factors only, was investigated although it would have been useful, time permitting, to examine other factors.

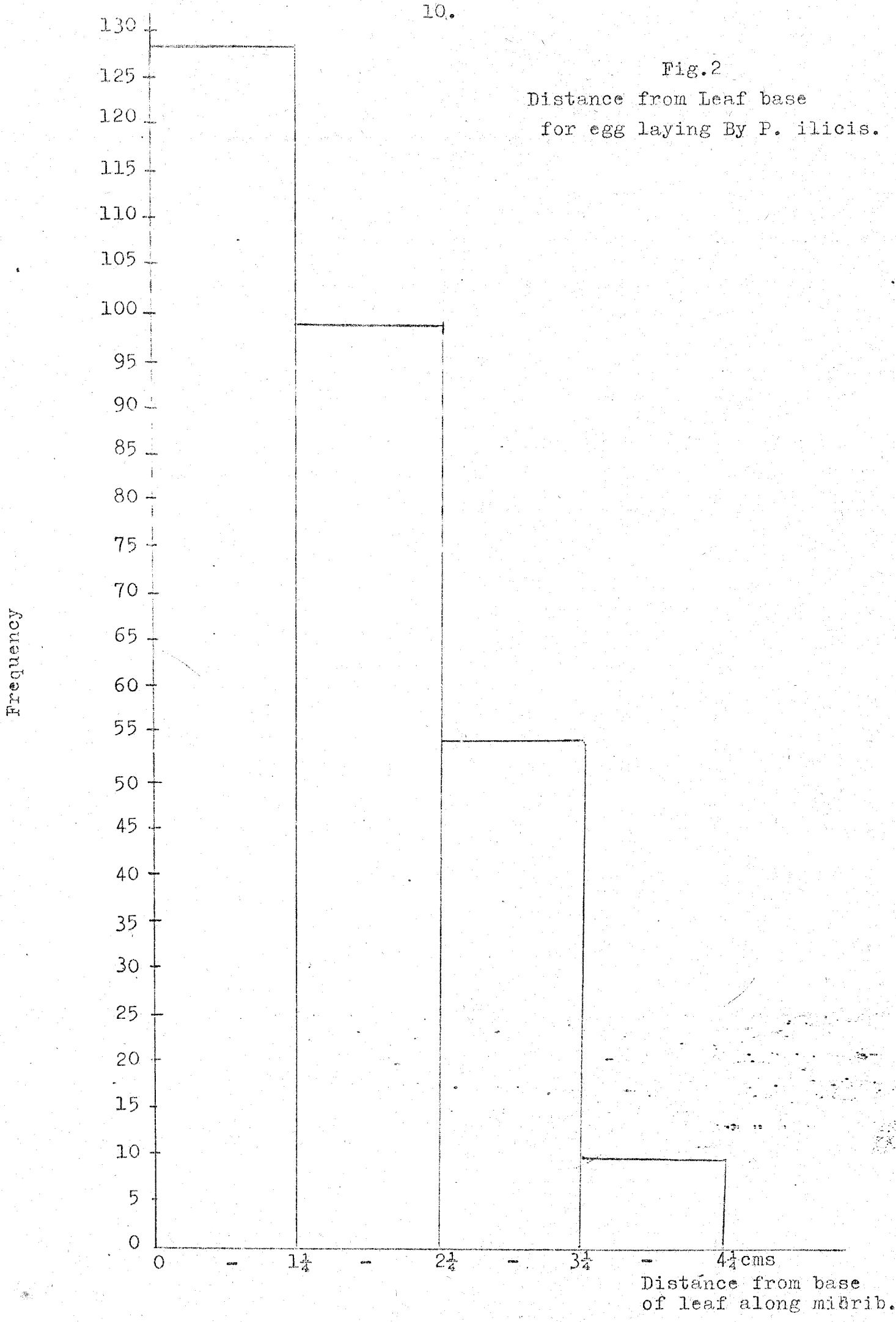
Later mortality factors, i.e. by bird-pecks and different species of parasites on P. ilicis were examined to determine whether a density-dependant relationship existed between percentage parasitism and the levels of infestation for each tree.

2.2 Life-history of Phytomyza ilicis

The life-history of the insect spans 1 year. The imago emerges from the puparium towards the end of May (Miall and Taylor 1907 Downes 1931), and after fertilization the female lays eggs on the young unthickened leaves of the current years growth. The foliage at this stage is non-waxy and soft, thus easily penetrated by the ovipositor. Pits were observed on the upper and lower surface of infected leaves only and are associated with feeding holes made during egg-laying.

The site where eggs are laid is located near the base of the midrib on the lower side of the leaves (Fig. 2). The female bores a vertical shaft which, on reaching the vessels of the leaf, bends at right angles and continues horizontally along the midrib. The egg is deposited in the horizontal section of the tunnel. The wound made in the midrib is closed by cork cells, hence it can be identified by running the thumb along the midrib. The egg is white and oval in shape measuring 0.383 by 0.160 mm. (Miall and Taylor 1907).

Fig.2
Distance from Leaf base
for egg laying By P. ilicis.



The egg hatches, but the first stage larva remains in the midrib until September - November, when it leaves the central vessels and enters the soft green outer tissues. The larva feeds on the parenchyma below the epidermis until large blotches appear on the leaf surface. The mine reaches maximum size in March, and usually occurs on the upper epidermis, although it can be found on both surfaces. Between July and March the larva moults twice passing through 3 larval stages, the first lasting from July to December, the second from December to January, and the third from February until the formation of the pupae late in March. Before pupating the mature larva prepares a thin triangular area on the cuticle of the leaf, against which a hinged emergence plate of similar size on the puparium will abut, so that escape by the mature fly will be easily accomplished.

Whilst feeding on the leaf tissues the larva lies on its side, but before entering the pupal stage it turns onto its back so that the vertical surface is pressed against the epidermis and its anterior spiracles are projected through the attenuated area of the cuticle. The imago makes its escape from the leaf by pressing the ptilinum against the hinged emergence plate on the puparium, which in turn breaks through the thin cuticle above it. (A detailed account is given by Miall and Taylor (1907).

2.3 Parasites of Phytomyza ilicis

Before 1939, only one previous record of a parasite of the holly leaf-miner was recorded - Dacnusa maculata, Gour (Braconid), on P. ilicis in Italy. Cameron (1939) was the first to record the listed species of parasites on P. ilicis.

Chalcidoidea

Eulophidae	1.	Chrysocharis gemma (Curt), Walk.
	2.	Chrysocharis syma, Walk.
	3.	Pleurotropis amyntas, Walk.
	4.	Closterocerus trifasciatus, Walk.
	5.	Tetracampa sp.
Pteromalidae	6.	Sphegigaster flavicornis, Walk.
	7.	Crytogaster vulgaris, Walk.
	8.	Eutelus sp.
Ichneumanoidea		
Braconida	9.	Opius ilicis, Nixon.

A key to the identification of all species at various stages of development is given by Cameron (1939). A brief discription and life-history of the parasites found during this investigation only will be given. Any supplementary information is given in detail in the forementioned paper.

a) Chrysocharis gemma (Curt), Walk.

This was observed to be the commonest parasite of the holly leaf-miner in the South of England. (Cameron 1939) 30-40% of fly larvae found during the investigation by Cameron (1937-1938), were attacked by this species. In 1938, at Burnham Beeches, Bucks. 71 from a 100 possible hosts were infected. The species is widely distributed throughout Europe, Africa, America and Australasia.

Female C. gemma have an unusual habit of overwintering in the adult stage. Egg laying occurs towards the end of February in Southern England. A small number of females may be found to oviposite as late as April, although few host larvae are available. A single egg is laid through the cuticle of the leaf, and is deposited in the body cavity of the larva. Superparasitism may occur but is infrequent.

Attacked larvae of P. ilicis become yellow and have a dirty pale yellow colour, which contrasts with the turgid bright shiny lemon colour of healthy larvae.

Incubation of the parasite eggs lasts from 7 - 10 days, according to temperature after which the first stage larvae appear. The larva is assumed to pass through at least three distinct stages and is mature after 16-20 days. After feeding is completed the larva remains in the resting stage for 8-10 days before the formation of prepupa, hence larval life lasts for approximately 4 weeks. Time spent in the prepupal and pupal stages is 4 and 38 days respectively. Cameron (1939) quotes pupae of C. gemma as being found from 12th April onwards. A description of the developmental stages is given by Cameron (1939).

b.) Chrysocharis syma, Walk.

C. syma is a parasite of the pupa and has only been recorded in England, Scotland and France.

When the fact that this parasite attacks the pupae only is considered, the general biology of the species is similar to that given for C. gemma. Eggs are laid internally in the fly pupae during April, depending on the availability of the latter. Larval development is similar to that of C. gemma; the prepupal and pupal stages lasting for 1½ and 19 days. Adult emergence was found to occur from early July onwards. Again a description of the developmental stages is given in detail by Cameron (1939).

c.) Sphegigaster flavicornis, Walk.

The species was found to be fairly well distributed throughout areas examined by Cameron. Its geographical range is North and Central Europe. A small number of host records are available and it appears that members of the genus Sphegigaster parasitize leaf mining and stem-boring Diptera, in addition to gall-making species of the family Cecidomyidae. A few species also attack aphids.

According to Cameron (1939), the parasite becomes active on the holly tree in early April. Oviposition is time consuming, since the insect must bore through the cuticle of the leaf and the tough skin of the puparium, and usually extends over 30 minutes. The egg is then deposited externally on the pupa of Phytomyza ilicis. Incubation lasts from 4 - 7 days concluding in the emergence of the first stage larva, which feeds externally on the host puparium. Five larval stadia exist. The larva feeds actively for 11 - 13 days, after which it enters a resting period spanning 5 days before it becomes a prepupa. The prepupal and pupal stages last for 2 days and 6 days respectively, and adults emerge from late June onwards although some individuals fail to complete metamorphosis until the middle of August.

In this investigation parasites were observed at the mature larval stage onwards. A key for classification of larval, prepupa and pupal stages is explained in Cameron (1939).

2.4 Sample area.

The area chosen for investigation runs parallel to Hollingside Lane, Durham city, G.R. NZ.276 405, and is part of the main Hollingside Wood. The approximate area is indicated on Fig. 3a. and spans 116 metres along the length of the lane, and 37 metres into the wood. The wood is deciduous, prominent species of trees being oak (Quercus patraea), beech (Fagus sylvatica), sycamore (Acer pseudo-platanus), silver birch (Betula pendula), with a few species of Scots Pine (Pinus sylvestris). Holly propagates both from seed and via under ground rhizomes, thus young bushes tend to spread laterally and are often clumped within a small area.

Sixteen holly bushes were chosen for examination on the basis of their isolation or proximaty to other holly bushes, position with respect to other vegetation, height and diameter, degree of healthiness based on the number of leaves per twig, sex and position with respect to gradient within the main wood. (Table 1.).

Trees examined were of varied morphology. Multi-stemmed structures were examined e.g. Tree 10, where three individual trees arose from a single main structure. Other bushes were small, having no prominent main structure but seemed to be formed from a mass of lateral branches. Under shade conditions branches tend to become pendulous, and on reaching the ground these form adventitious roots, thus root suckers are common e.g. Trees 4 and 7. The older hence taller trees tended to have fewer leaves per branch at higher levels, the former having fewer teeth per leaf, and these were observed to be less shiny when compared with leaves taken from lower levels. Aspect was also found to influence the number of leaves found per branch.

Thus trees examined varied in growth form and position with respect to the lane. Those nearest Hollingside Lane were assumed to suffer more from exposure since they were not as sheltered as trees within the wood itself. However they were overshadowed to a lesser degree by dominant vegetation. After 6 metres, the ground sloped steeply downhill, (1 : 2 gradient), hence trees examined on these sites (3,4,5,6,7,8,10,15,16) experienced differing physical conditions - leaching, water availability - from those situated at the top of the slope (1,2,11,12,13,14).

Six suction-traps were set up in a group of three adjacent trees, situated on sloping terrain, within Durham University field-station (Fig 3a.), at varying heights above ground level to monitor the emergence of P. ilicis adult flies of the 1977 season, and those adult flies during egg-laying. The traps were also intended to monitor the number of parasitic flies of Phytomyza ilicis.

Details for each tree sampled and their proximaty to each other is given in Fig. 3b, Table 1.

Table 1. Description of Trees sampled.

TREE NO.	HEIGHT (METRES)	DIAMETER	LENGTH	DISTANCE FROM LANE TO TREE	DISTANCE BETWEEN TREES	DESCRIPTION OF OF NEIGHBOURING VEGETATION
1.	3.70	2.60	1.80	5.80	1-2, 15.70	OAK, BEECH, WILLOW
2.	2.80	2.50	2.50	4.00	2-3, 7.50	OAK
3.	5.70	7.60	8.00	14.80	3-4, 19.50	OAK, S. BIRCH, PINE
4.	2.80	4.00	6.00	18.40	4-5, 6.20	OAK, S. BIRCH
5.	1.70	4.00	3.85	12.20	5-6, 15.50	OAK, S. BIRCH, PINE
6.	2.90	4.40	4.20	21.00	6-6, 3.00	OAK, BEECH
7.	2.40	3.10	3.25	19.40	7-8, 13.00	BEECH, S. BIRCH
8.	4.35	3.10	4.35	5.00	8-9, 5.00	OAK, BEECH
9.	2.85	3.90	3.50	4.00	9-10, 3.00	OAK
10.	5.55	7.00	11.40	7.50	10-11, 6.40	OAK, S. BIRCH
11.	2.35	3.10	2.70	20.90	11-12, 3.50	BEECH, SYCAMORE
12.	9.60	5.30	4.80	9.50	12-13, 5.50	BEECH, OAK
13.	2.70	3.40	3.35	11.70	13-14, 3.20	OAK
14.	7.40	6.85	6.60	14.00	14-15, 7.00	OAK
15.	5.60	5.40	7.30	30.00	15-16, 2.75	BEECH , OAK.
16.	6.59	7.40	6.40	37.00		BEECH.

All measurements in metres.

Fig 3a. Map of main sampling area.

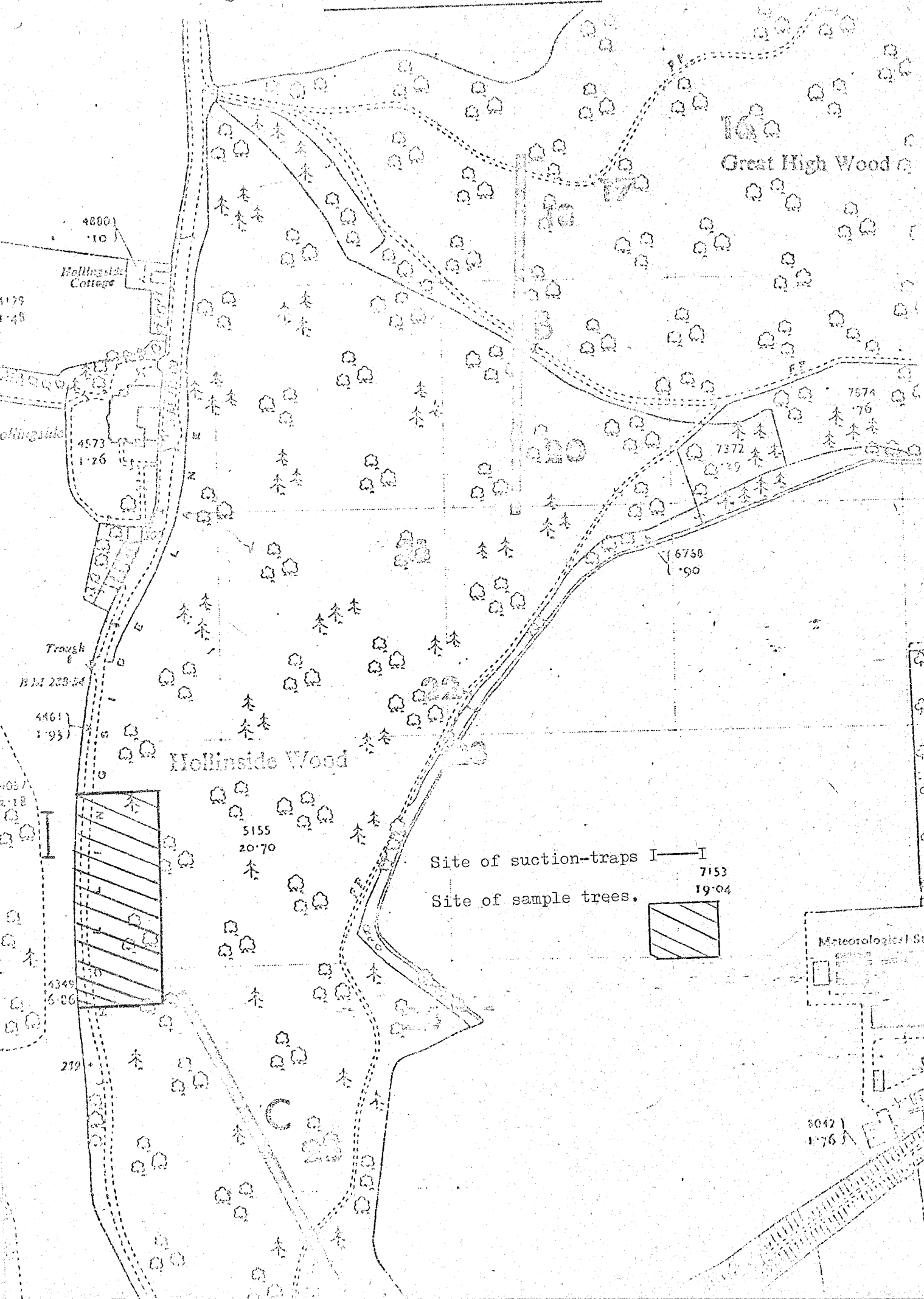
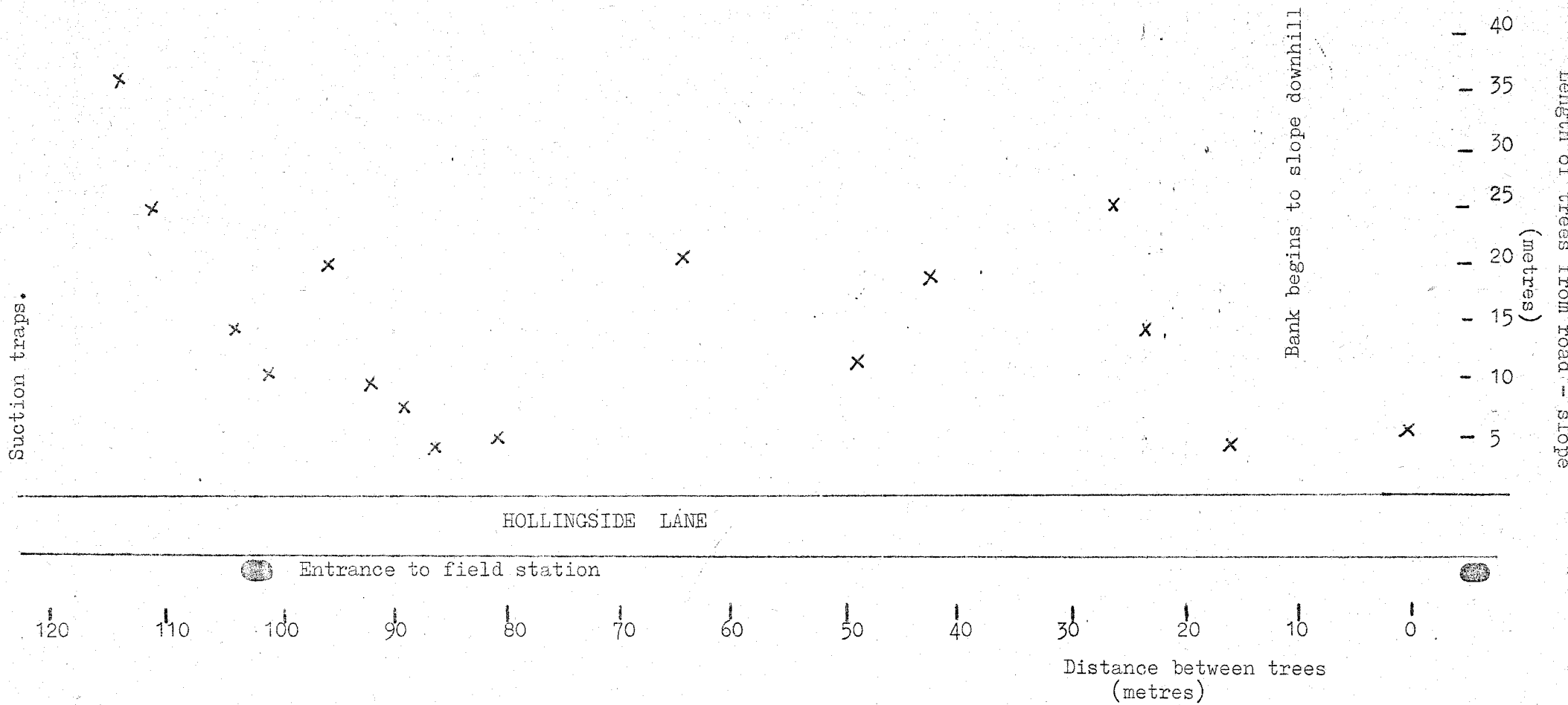


FIG. 3,B.

Map of tree distribution within the area of investigation outlined in Fig. 3 (a)



Length of trees from road - slope

3. Method
3.1. Sampling

The aim of the investigation was to measure the level of infestation within a defined area, thus trees examined had to be representative of the size and age range of Ilex aquifolium within the area. The size of bushes and trees examined varied between 1.70 and 9.60 metres, and this coupled with the irregular growth pattern of Ilex posed a major problem with regard to choosing a suitable sampling method, since one method was not applicable to all trees.

An initial sample of 100 leaves was taken from each tree to estimate the level of infestation. Stokes (1969), estimated a minimum sample size of 750 leaves, concluding that this estimate would lie within 10% of the correct figure for infestation. In this investigation the effect of parasitism was investigated and since percentage parasitism was found to vary between trees, it was necessary to sample as many leaves as was practical (i.e. 325 - 4300 leaves depending on the size of the tree). Collection of data was carried out from mid-April until late July, thus only the latter half of the complete growth cycle was examined for 1977 season and the begining of the 1978 season.

Variation in the level of infestation with aspect and height was investigated and is discussed later. Assuming no significant variation in infestation with both aspect and height, then a random sampling method can be adopted. However should a difference between different areas of the same tree be found, then ideally more leaves should be sampled from areas indicating lowest infestation. It is extremely difficult to obtain a genuinely random sample of shoots or leaves and in this study population density is expressed as the value per 100 leaves sampled. As the number of leaves sampled for each tree varied, this was a good method for making comparisons between trees. To get an absolute estimate of population density is difficult since the total number of leaves on the tree is required and this posed another unsolved sampling problem, which is discussed in detail later.

Various sample sizes were taken for earlier trees examined to estimate the minimum sample size required and the variation in accuracy with increased sampling effort. Results are discussed later.

Initially for Tree 1. 42 twigs of similar length were sampled to investigate variation in levels of infestation with aspect and 6' twigs were also sampled per 50 cm height interval to investigate height variation. Although these twigs were of similar length, the number of leaves supported by each twig varied and posed problems with data analysis. Thus the number of twigs sampled per tree was reduced and the final number of leaves per sample was kept constant for ease in statistical analysis. The method used was simple. Twigs were sampled at regular intervals, starting from the base of the bush and sampled randomly over the entire bush to the apex. The final number of leaves obtained per tree depended on the size of the sample tree.

All leaves sampled were examined for the presence of eggs, and egg numbers per leaf were recorded. The position of eggs along the midrib was also measured. Presence or absence of a mine on a leaf was recorded, together with the number of mines supported by the leaf and their position, i.e. whether on the adaxial or abaxial leaf surface. Each mine was examined for bird attack characterized by a V-shaped peck-mark within the mine, for small triangular emergence holes characteristic of adult P. ilicis and for small round emergence holes characteristic of the adult parasites of the leaf-miner, (Lewis and Taylor, 1974). Opened mines were examined and contents identified under a binocular microscope. Thus it was possible to determine the number of adults which had emerged successfully, the number of individuals, i.e. both mature larvae and pupae which were parasitized, the species of parasite present, the extent of development and finally the number of individuals which had died from undetermined factors. Mines from which C. gemma, the larval parasite had emerged, were characterized by the absence of the orange pupal case of P. ilicis , coupled with the black shiny remains of the Chrysocharis pupal case.

Individuals extracted from the mines were also measured.

A 100 leaves per tree were sampled for the new season's growth and the number of eggs per 100 leaves determined.

3.2 Suction-traps.

Six suction-traps, were established at three height intervals, 1,3 and 6 metres above ground level, within a clump of three holly trees within the field-station site (Fig. 3a), and operated from two battery chargers. These were emptied at weekly intervals throughout the investigation and the contents examined. The traps were set up during the middle of April, when adult C. gemma are abundant and continued running until late July, when it was hoped to sample all adult parasites and adult P. ilicis. Insects were identified to family level initially but detailed examination of trap contents was too time-consuming, thus trap contents were only examined for leaf-miners and their parasites.

3.3 Leaf-section.

The level of infestation by P. ilicis is known to fluctuate between sample trees, and since it was one of aims of the investigation to determine possible causes for this variation, both leaf and cuticle thickness for Ilex aquifolium leaves was measured.

A random sample of 7 leaves from 1977 season, which had been mined and a further sample of 7 leaves without mines were obtained for each tree, so that a total of 14 leaves were taken for the entire tree. Obviously cuticle thickness will have increased since the eggs were first laid, but it was hoped that any variation in both leaf and cuticle thickness would still exist between trees.

An initial sample of leaves from a single holly tree was taken to determine whether any relationship between size, leaf thickness and cuticle thickness for both the upper and lower epidermis existed. Size was found to influence both leaf and cuticle thickness, (section 4.4), thus the size of sampled leaves for all trees was kept as uniform as possible. During the current seasons growth, leaves of various sizes were sampled from the same tree to monitor cuticle development, since it is at this stage that leaves are attacked by P. ilicis.

For all leaves sampled a thin section was taken across the midrib (Fig.17) and the thickness of the leaf at sites a,b,c were measured. Initially cuticle thickness was measured at the three points, but little variation between a and c was found, thus measurements were reduced to a and b. A transverse section was prepared for each leaf as follows and mean values for leaf and cuticle thickness obtained for each tree.

T.S. sections :-

All sections were fixed in Rawlins fluid overnight. They were then immersed in 70% alcohol for approximately 1 hour, after which they were transferred to 95% and two changes of absolute alcohol respectively, for 1 hour duration with each change of fluid. The sections were then placed in a 1:1 alcohol/xylene fluid for 1 hour, followed by two changes of pure xylene for 1 hour with each change of fluid. Sections were transferred to a xylene/wax mixture at 57°C for 1 hour. Three changes of fresh wax was used over a 12 hour period. The third change was left overnight. The tissues were then blocked in fresh wax containing 0.5% ceresin and left to harden. 7.5 μ sections were cut on a microtome and floated on warm water to flatten the wax-sections. Each group of leaves were placed on a glass slide, which had been spotted with egg albumin/thymol mixture and smeared over the slide to prevent clouding of the final tissue-section. The slides were dried on a hot-plate at 37°C.

Finally the immersion process was reversed for each slide, each change of fluid lasting for 1 hour. The slides were then immersed in water and stained with 1% aqueous safranin. The staining process was taken back to the xylene stage and each section mounted in a D.P.X. mountant and left to harden.

Each section was measured for leaf thickness at X40 magnification and for cuticle thickness at X400. All values were converted to cms.

4. Discussion of results.

4.1 Data for individual trees.

4.1.1 Problems with sample size.

When Stokes (1969), carried out his investigation on Phytomyza ilicis for Ancient Camp area of North Wales, the sampling method adopted was extremely complex and resulted in 100 - 150 leaves being sampled for each site. In this investigation the accuracy associated with a small sample size, and that of a larger sample was compared for each tree. The problem with a small sample size, apart from being unrepresentative of the entire tree, is that a small increase in total egg or mine number per hundred leaves sampled, results in a considerably higher estimate of infestation. Phytomyza mines are clearly visible thus from a small sample, levels of infestation for the entire tree tend to be overestimated as a result of subjective errors, hence by using large samples for overall estimates of infestation, and by sampling twigs of similar size only, it was hoped to reduce the amount of subjectivity.

The physical description of each tree examined together with proximate vegetation is given in Table 1, and the extent of isolation of each tree is indicated in Fig. 3b. From a theoretical viewpoint the proximaty of trees to each other was assumed to influence the level of infestation of individual trees, the reasoning being that trees closer together are more likely to be reinfected by adult Phytomyza from adjacent trees, whilst trees further from the main concentration of adult flies prove less favourable hosts, since more time is wasted travelling between trees and searching for suitable oviposisition sites. Measurements of height and diameter for the sample trees indicate both age and growth vigour, and this may also influence levels of infestation, since healthy trees presumably provide a more favourable enviroment for the completion of the life-cycle .

Trees sampled can be catergorised on the basis of position within the wood; i.e. those situated on the edge of the wood at the top of the bank (Trees 1,2,11,13,14), those intermediatery in position (Trees 3,5,8,9,10), and finally those at the base of the slope positioned within the wood itself.

A summary of the number of samples and sample size is given in Table 3 . The results based on 100 leaves sampled is given in Table 4 , Fig.4 . Generally a bigger sample size reduces the estimated values for egg density, total mines observed and that for infestation. Exceptions to this are estimates for Trees 6 and 7, where values increased with sample size. A summary of results for all parameters investigated for 16 trees, together with the total number of leaves sampled is given in Table 5 . Large deviations in estimates for all parameters were observed for Trees 2,6,7,9,11, according to the number of leaves sampled.

For Tree 1, Table 3 and 4 , three samples were taken, i.e. 100, 1,384 and 3,108 leaves, and as for all results, the number of leaves with mines, total number of mines, total number of eggs was determined. These values were expressed per 100 leaves, in order that comparisons between samples and ultimately between trees, could be made.

Table 3.
3a) Tree 1.

Summary of results for 2 different sample sizes :-

TOTAL NO. OF LEAVES	NO. LEAVES WITH MINES	TOTAL NO. OF MINES	TOTAL NO. OF EGGS	MINES -1 100 LEAVES	EGGS -1 100 LEAVES	INFESTATION	SE	INFESTATION + SE
3108	327	340	417	10.94	13.42	10.36	0.5	10.86 - 9.86
1384	160	168	195	12.14	14.09	11.56	2.38	13.94 - 9.18

3b) Tree 2.

Summary of results for 2 different sample sizes :-

TOTAL NO. OF LEAVES	NO. LEAVES WITH MINES	TOTAL NO. OF MINES	TOTAL NO. OF EGGS	MINES -1 100 LEAVES	EGGS -1 100 LEAVES	INFESTATION	SE	INFESTATION + SE
900	65	68	81	7.56	9.00	7.22	0.8	8.02 - 6.42
1384	121	124	149	8.96	10.77	8.74	0.9	9.63 - 7.83

3c) Tree 3.

Summary of results for 3 different sample sizes :-

TOTAL NO. OF LEAVES	NO. LEAVES WITH MINES	TOTAL NO. OF MINES	TOTAL NO. OF EGGS	MINES -1 100 LEAVES	EGGS -1 100 LEAVES	INFESTATION	SE	INFESTATION + SE
1403	224	230	300	16.39	21.38	15.97	0.98	16.95 - 14.99
1600	261	269	360	16.81	22.5	16.31	0.92	17.23 - 15.89
4300	767	804	1105	18.60	25.70	17.84	0.58	18.42 - 17.26

3d) Tree 6.

Summary of results based on 2 sample sizes :-

TOTAL NO. OF LEAVES	NO. LEAVES WITH MINES	TOTAL NO. OF MINES	TOTAL NO. OF EGGS	MINES -1 100 LEAVES	EGGS -1 100 LEAVES	INFESTATION	SE	INFESTATION + SE
453	101	108	140	23.84	30.91	22.30	1.96	24.26 - 20.34
1076	251	272	345	25.28	32.06	23.33	1.28	24.62 - 22.05

3e) Tree 7.

Summary of results for 3 different sample sizes :-

TOTAL NO. OF LEAVES	NO. LEAVES WITH MINES	TOTAL NO. OF MINES	TOTAL NO. OF EGGS	MINES -1 100 LEAVES	EGGS -1 100 LEAVES	INFESTATION	SE	INFESTATION + SE
325	61	62	77	19.08	23.70	18.77	2.17	20.94 - 16.60
107	25	26	32	24.30	29.91	23.36	4.09	27.45 - 19.27
218	36	36	45	16.51	20.60	16.51	2.51	19.02 - 14.00

Table 4.....

SUMMARY OF RESULTS FOR ALL TREES BASED ON 100 LEAVES SAMPLED

Tree No.	Mines per 100 Leaves	Eggs per 100 Leaves	Infestation S.E.
1	15.00	17.00	15.00 18.35 11.65
2	17.00	18.00	17.00 20.90 11.20
3	15.00	18.00	15.00 18.35 11.65
4	16.82	17.76	15.5 16.85 15.20
5	29.66	37.29	27.00 32.5 23.00
6	20.39	26.21	18.5 22.00 14.5
7	24.30	29.91	23.5 27.00 19.5
8	9.62	10.58	8.8 12.6 6.5
9	21.00	27.00	21.00 21.84 14.16
10	30.00	45.00	26.00 30.5 21.5
11	18.00	24.00	17.00 20.76 13.24
12	18.00	28.00	16.00 20.12 11.88
13	12.00	13.00	12.00 15.24 8.76
14	25.00	38.00	24.00 28.27 19.73
15	14.00	16.00	13.00 16.36 9.64
16	14.00	21.00	12.00 15.25 8.75

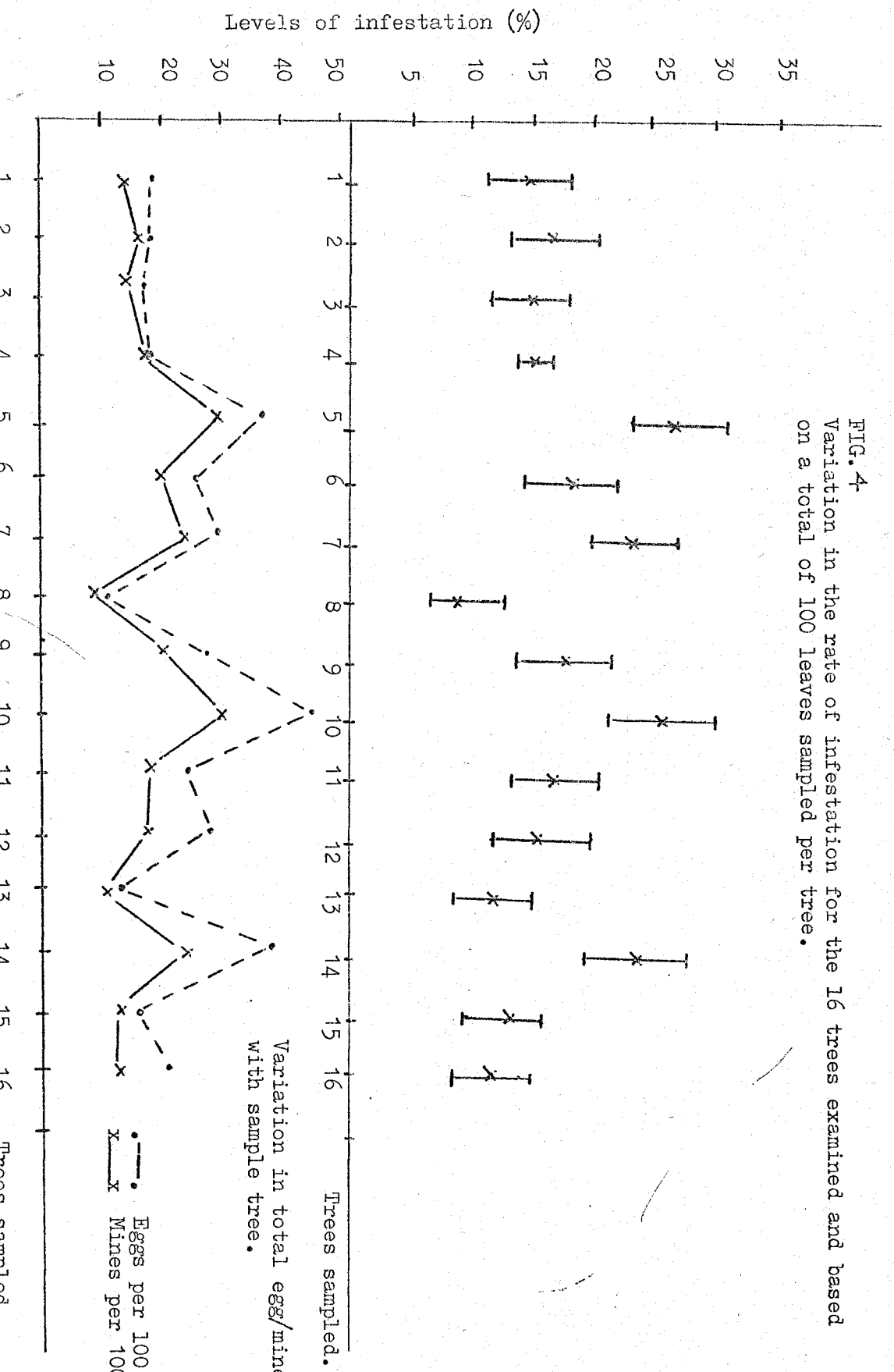


FIG. 4
Variation in the rate of infestation for the 16 trees examined and based on a total of 100 leaves sampled per tree.

Table 5.

TOTAL NO. OF LEAVES	NO. OF LEAVES WITH MINES	TOTAL NO. OF MINES	TOTAL NO. OF EGGS	MINES 100 -1 LEAVES	EGGS 100 -1 LEAVES	INFESTATION	SE	INFESTATION + SE	TREE NO.
3108	327	340	417	10.94	13.42	10.36	0.5	10.86- 9.86	1
1384	121	124	149	8.96	10.77	8.74	0.9	9.63 - 7.83	2
4300	767	804	1105	18.60	25.70	17.84	0.58	18.42-17.26	3
1491	207	219	277	14.69	18.58	13.88	0.895	14.70-12.99	4
1718	371	411	571	23.93	33.24	21.59	0.1	21.69-21.49	5
1076	251	272	343	25.28	32.06	23.33	1.28	24.62-22.05	6
325	61	62	77	19.08	23.70	18.77	2.17	20.94-16.60	7
703	65	67	99	9.53	14.08	9.20	1.09	10.29- 8.11	8
1400	117	186	262	13.29	18.71	12.64	0.89	13.53-11.85	9
2700	542	617	847	22.37	31.37	20.07	0.78	20.84-19.30	10
674	63	67	88	9.94	13.06	9.35	1.12	10.47- 8.23	11
847	167	182	262	21.49	30.93	12.72	1.37	21.09-18.35	12
714	69	90	113	12.61	15.83	9.66	0.68	10.34-8.98	13
1113	231	245	314	22.01	28.21	20.75	1.20	21.95-19.35	14
776	203	227	312	29.25	40.21	26.16	1.58	27.74-24.6	15
402	63	83	91	20.65	20.89	15.00	1.78	16.78-13.2	16

Estimates of infestation for Tree 1 with 100 leaves was 15 ± 3.35, for 1,384 and 3,108 leaves, estimates were 11.56 ± 2.38 and 10.36 ± 0.5 respectively. Thus for a X 300 fold increase in leaves sampled estimates for infestation were reduced by 33%. Considering results for 100 leaf-sample and 1,000 leaf-sample, no difference in the overall level of infestation was found since the confidence intervals overlapped.

For Tree 2, three samples were taken but were of 100,900 and 1,384 leaves. Values for infestation again were varied, 17.0 ± 3.76, 7.22 ± 0.8 and 8.74 ± 0.89 respectively. Thus a X9 fold increase in sample size reduced the estimate by 41%. Little difference was found betwwen 900 and 1,384 leaf-samples.

Four samples were taken for Tree 3. These were of 100, 1,403, 1,600, and 4,300 leaves as given in Table 3 and 4 . Again variation was observed with sample size. For 100 leaves, estimates were 15.0 ± 3.35, for 1,403 leaves - 15.97 ± 0.98, for 1,600 leaves 16.31 ± 0.98, and finally for 4,300 leaves - 17.84 ± 0.58. Thus for Tree 3, excluding the results for small sample size, increasing the number of leaves sampled from 1403 to 4,300 improved the estimate to such a small degree when maximum values were considered, that under normal sampling proceedures, it appears 1,000 leaves are sufficient to give reliable estimates. In this investigation, an idea of the number and also species of parasites attacking Phytomyza was required, thus it was necessary to sample as many leaves as was practical in terms of time and effect upon the host tree.

For Tree 6, a X2½ fold increase in sample size had little influence on the overall estimates for infestation, however the errors involved with too small a sample size is indicated for Tree 7, Table 3e.

Thus the ideal minimum samples size for all trees was taken as 800 - 1,000 leaves where the size of the trees permitted. The sample size taken for Tree 16 was too low for an accurate estimate. A more realistic figure would be approximately 10%. Ideally more leaves should have been collected.

4.1.2. Summary of results for all parameters investigated.

The results for total egg - density, mine number, and overall infestation for the 16 sample trees is given in Table5 . Using Chi-square tests the differences for all parameters between trees was investigated for significance. When values for infestation were compared X^2 was found to be 30.2. This was significant at 5% level. The result was insignificant at the 1% level. The trees were ranked according to size and tested using a 2X2 contingency - test, to determine whether height of trees was the factor causing the observed variation. Results were insignificant at 5% level. Thus the overall levels of infestation are independant of height and it was assumed independant of the diameter of bushes although this was not tested.

Calculated X^2 values for egg-density variation between trees and total mine variation gave values of 50.24 and 33.85 respectively. Both results were significant at 5% and 1% level of significance. From Table 5 , using egg-data as estimates of population density, Phytomyza density is highest for Trees 5, 6, 10, 12 and 15. Those least affected being Trees 1,2,8 and 13. Lowest values were observed for Trees 1 and 2, and these were the most isolated (Fig. 3b). Trees 4 and 5 were clumped into a small area, similarly for Trees 6 and 7, with approximately 15 metres between these areas. Egg-density estimates were close for Trees 4 and 5, but not so for 6 and 7. Trees 8 - 14 were clumped within a restricted area thus assuming the previous hypothesis is correct egg-density was expected to be higher within this area. When mean values were compared for the three main areas, egg-density was greatest for units 6 - 7, thus although isolated trees tended to have lower population density of Phytomyza little difference was observed between moderately spaced trees and those within a restricted space.

From Tables1 and5 it would appear trees nearest the road have lower levels of infestation compared with those further into the wood. Areas where greatest difference between egg-density existed were for those trees at the top of the slope and those situated on the slope. Comparisons of the mean values for egg-density gave t - 1.06 at 5% level of significance. The tabulated value was 2.31, therefore the difference was insignificant.

Trees 3 and 10 were female trees, identified by the red berries from the previous season, and these were found to have high values for both total egg number and mine number. However no conclusions could be drawn since it was very difficult to sex the remaining trees. Absence of berries was not an accurate indication of sex, hence for discrimination between trees, more effort should have been put into sexing them prior to the investigation.

4.1.3 Aspect variation.

Investigation into possible variations in the level of infestation with aspect was carried out for Trees 1,2,3,4,5, and 9, i.e. over the size range of trees sampled.

For Tree 1, Table6,42 twigs were sampled per side of the tree for all heights and these were of similar lengths. However the number of leaves per twig varied. Those on the East-facing side were more sparse probably due to competition for light with neighbouring vegetation. Leaf number on the West-facing side was highest hence growth was more vigourous. Thus equal numbers per side of the tree were ultimately sampled. The size for Tree 1 was 346 leaves since this was the maximum possible for the Eastern aspect without causing damage to the tree. (Table 6b). Comparisons for the different sample sizes are indicated in Table 6c , the error involved with reduced sample size being slight except for results from West-side, where sample size had been reduced by 69%. The calculated value for X^2 to test for differences in infestation with aspect, using data for total mine numbers was 6.40 for 3 degrees of freedom at the 5% level of significance (Appendix 2.). This was lower than tabulated values for X^2, hence no significant difference resulted, although from Table 6c, both infestation and total mine density appeared to be higher on East and South-facing sides.

Table 6.
Tree 1. 6a.
Variation in level of infestation by Phytomyza ilicis with aspect
Sample based on 42 twigs of similar length :-

ASPECT	TOTAL NO. OF LEAVES	LEAVES WITH MINES	TOTAL NO. OF MINES	TOTAL NO. OF EGGS	EGGS -1 100 LEAVES	MINES -1 100 LEAVES	SE.	INFESTATION + SE
E	467	76	77	98	20.99	16.49	1.70	16.27 $<^{16.97}_{14.50}$
W	1115	92	96	115	10.31	8.60	0.82	8.25 $<^{9.07}_{7.33}$
N	667	55	60	67	10.04	8.99	1.0	8.24 $<^{9.24}_{7.24}$
S	859	99	107	137	15.95	12.48	1.0	11.55 $<^{12.55}_{10.55}$

Tree 1. 6b.
Constant sample size :-

ASPECT	TOTAL NO. OF LEAVES	LEAVES WITH MINES	TOTAL NO. OF MINES	TOTAL NO. OF EGGS	EGGS -1 100 LEAVES	MINES -1 100 LEAVES	SE.	INFESTATION + SE.
E	346	54	55	67	19.36	15.90	1.95	15.61 $<^{17.56}_{13.66}$
W	346	37	38	40	11.56	10.98	1.66	10.69 $<^{12.35}_{9.03}$
N	346	29	32	35	10.12	9.25	1.98	8.38 $<^{10.36}_{6.04}$
S	346	40	43	53	15.32	12.43	1.72	11.56 $<^{13.28}_{9.84}$

Tree 1. 6c.
Summary for the 2 sample sizes

EGGS -1 100 LEAVES	MINES -1 100 LEAVES	INFESTATION	SE	INFESTATION + SE.	TOTAL NO OF LEAVES	ASPECT
20.99	16.49	16.27	1.70	16.97 - 14.50	467	E
19.36	15.90	15.61	1.95	17.56 - 13.66	346	
10.31	8.60	8.25	0.82	9.07 - 7.33	1115	W
11.56	10.98	10.69	1.66	12.35 - 9.03	346	
10.04	8.99	8.24	1.0	9.24 - 7.24	667	N
10.12	9.25	8.38	1.9	10.36 - 6.40	346	
15.95	12.48	11.55	1.0	12.55 - 10.55	859	S
15.32	12.43	11.56	1.72	13.28 - 9.84	346	

A significant difference however in the number of eggs laid per 100 leaves with aspect was observed. The calculated X^2 was 13.1 compared with a tabulated value of 7.81. This was significant at both 5 and 1% levels (Appendix2). Fewer eggs were laid on Noth-facing leaves, maximum values for leaves on Eastern-facing areas, possibly because the latter is more sheltered.

For Tree 2 the final sample size was 346 per side of the tree. Comparisons in the level of infestation for total mine data gave a significant difference with aspect at 5 and 1% levels of significance, (Table7b) (Appendix2). For egg density data, a significant difference was observed at both levels of significance (Appendix2). Maximum eggs were laid on East-facing leaves, however fewest eggs were found on Westerly leaves. Total mine data gave a similar result, North and South-facing leaves having similar levels of infestation (Table7c).

For Tree 3, 400 leaves were sampled per side of the tree and the variation with aspect is indicated in Table8b. Results for both egg density and total mine number were significant at both 5 and 1% levels of significance (Appendix 2). However infestation was lowest on the Eastern-side i.e. 11.00 $\pm$ 1.5, and highest on the Western-face i.e. 22.50 $\pm$ 2.08. Corresponding values for eggs per 100 leaves were 14.50 and 32.00.

For these three trees a comparison between the results based on a constant sample of twigs and that for constant leaf number were compared, (Tables 6c, 7c, 8_c). A sample size of 300 - 400 leaves were taken as the most practical.

For Tree 4, the total sample size was 346 leaves per side of the tree (Table9). Values for X^2 for both egg-density and total mine number gave significant result. For total mine number the calculated X^2 was 18.17 compared to a tabulated value of 7.81, and that for egg-density was 31.43 compared with 7.81. These were significant even at the 1% level (Appendix 2). In terms of egg-density data, the lowest population densities were observed for East and South-facing sides, maximum for the West side. This trend was observed for total mine data.

For Tree 5, the sample size was 400 leaves,(Table10). An insignificant differences at the 5% level was observed for total mine data (Appendix2), i.e. calculated value for X^2 was 7.19, compared with a tabulated value of 7.81. For egg-density data, the result was significant at both 5 and 1% levels i.e. calculated value for X^2 was 15.22 compared with a tabulated value of 7.81. Differences between egg number was small for East, North and Southern aspects; lowest values appearing for the West side. Similar trends were observed with total mine data.

For Tree 9, a total sample size of 300 leaves per side of the tree was used (Table11). No significant difference was observed for both egg-density and total mine number (Appendix 2) although from raw data, Eastern areas had higher values for both egg and mine number.

Tree 2 7a.
Variation in level of infestation by Phytomyza ilicis with aspect.
Sample based on 22 twigs of similar length

ASPECT	TOTAL NO. OF LEAVES	LEAVES WITH MINES	TOTAL NO. OF MINES	TOTAL NO. OF EGGS	EGGS $^{-1}$ 100 LEAVES	MINES $^{-1}$ 100 LEAVES	SE	INFESTATION + SE
E	171	17	19	26	15.20	11.10	2.28	$9.94 < {}^{12.22}_{7.66}$
W	295	11	12	13	4.06	4.06	1.14	$3.87 < {}^{5.01}_{2.73}$
N	216	15	15	18	8.33	6.90	1.72	$6.09 < {}^{8.62}_{5.18}$
S	218	22	22	24	11.01	10.09	2.13	$10.09 < {}^{12.22}_{7.96}$

Tree 2. 7b.
Constant sample size :-

ASPECT	TOTAL NO. OF LEAVES	LEAVES WITH MINES	TOTAL NO. OF MINES	TOTAL NO. OF EGGS	EGGS $^{-1}$ 100 LEAVES	MINES $^{-1}$ 100 LEAVES	SE	INFESTATION + SE.
E	346	46	48	65	18.79	13.87	1.82	$13.29 < {}^{15.11}_{11.47}$
W	346	17	18	20	5.78	5.20	1.16	$4.91 < {}^{6.07}_{3.08}$
N	346	29	29	34	9.83	8.47	1.94	$8.47 < {}^{10.41}_{6.53}$
S	346	29	29	30	8.67	8.38	1.49	$8.38 < {}^{9.87}_{6.89}$

Tree 2. 7c.
Summary for the 2 sample sizes

ASPECT	TOTAL NO. OF LEAVES	EGGS -1 100 LEAVES	MINES -1 100 LEAVES	INFESTATION	SE.	INFESTATION + SE.
E	171 346	15.20 18.78	11.10 13.87	9.94 13.29	2.28 1.82	12.22 - 7.66 15.11 -11.47
W	295 346	4.06 5.78	4.06 5.20	3.87 4.91	1.14 1.16	5.01- 2.73 6.07- 3.8
N	216 346	8.33 9.83	6.9 8.47	6.9 8.47	1.72 1.94	8.62- 5.18 10.41- 6.53
S	218 346	11.01 8.67	10.09 8.38	10.09 8.38	2.13 1.49	12.22- 7.96 9.87- 6.89

Table 8. 8A.
Variation in level of infestation by P. ilicis for tree 3, with aspect.
Sample based on 22 twigs of similar length

ASPECT	TOTAL NO. LEAVES	LEAVES WITH MINES	TOTAL NO. OF MINES	TOTAL NO. OF EGGS	EGGS -1 100 LEAVES	MINES -1 100 LEAVES	SE.	INFESTATION + SE.
E	321	33	36	45	14.10	11.20	1.70	10.28 < 11.98 / 8.58
W	354	77	79	106	29.90	23.30	2.0	21.47 < 23.47 / 19.47
N	398	59	59	79	19.85	14.80	1.82	14.80 < 16.62 / 13.02
S	300	55	56	70	21.20	16.97	2.03	16.67 < 18.70 / 14.64

Table 8 8b.
Constant sample size

ASPECT	TOTAL NO. LEAVES	LEAVES WITH	TOTAL NO. OF MINES	TOTAL NO. OF EGGS	EGGS -1 100 LEAVES	MINES -1 100 LEAVES	SE.	INFESTATION + SE.
E	400	44	47	58	14.50	11.75	1.5	11.00 < 12.5 / 9.5
W	400	90	94	128	32.0	23.5	2.08	22.5 < 24.58 / 20.42
N	400	59	59	80	20.0	14.75	1.78	14.75 < 16.53 / 12.97
S	400	68	69	94	23.50	17.25	1.88	17.00 < 18.88 / 15.12

Table 8 8c.
Summary for 2 sample sizes

EGGS -1 100 LEAVES	MINES -1 100 LEAVES	INFESTATION	SE.	INFESTATION + SE.	TOTAL NO. OF LEAVES	ASPECT
14.1 14.25	11.21 10.75	10.28 10.00	1.70 1.50	11.98-8.58 11.50-8.50	321 400	E
29.9 31.5	22.3 23.5	21.47 22.50	2.0 2.08	23.47-19.47 24.58-20.42	354 400	W
19.85 19.75	14.80 14.75	14.80 14.75	1.82 1.78	16.62-13.02 16.53-12.97	398 400	N
21.20 21.25	16.97 17.25	16.67 17.00	2.03 1.88	18.7 -14.64 18.88-15.12	330 400	S

Table 9.
Variation in level of P. ilicis for Tree 4 aspect.
Constant sample size :-

ASPECT	TOTAL NO. LEAVES	LEAVES WITH MINES	TOTAL NO. OF MINES	TOTAL NO. OF EGGS	EGGS $^{-1}$ 100 LEAVES	MINES $^{-1}$ 100 LEAVES	SE.	INFESTATION + SE.
E	346	37	39	51	14.74	11.27	1.66	$10.70 < \begin{matrix} 12.36 \\ 9.04 \end{matrix}$
W	346	72	76	103	29.77	21.97	2.18	$20.14 < \begin{matrix} 22.36 \\ 18.10 \end{matrix}$
N	346	42	46	57	16.47	13.29	1.76	$12.14 < \begin{matrix} 13.90 \\ 10.38 \end{matrix}$
S	346	39	40	47	13.58	11.56	1.70	$11.27 < \begin{matrix} 12.97 \\ 9.57 \end{matrix}$

Table 10.
Variation in level of infestation with aspect for Tree 5.

ASPECT	TOTAL NO. LEAVES	LEAVES WITH MINES	TOTAL NO. OF MINES	TOTAL NO. OF EGGS	EGGS $^{-1}$ 100 LEAVES	MINES $^{-1}$ 100 LEAVES	SE.	INFESTATION + SE.
E	400	80	89	130	32.50	22.50	2.00	$20.00 < \begin{matrix} 22.00 \\ 18.00 \end{matrix}$
W	400	69	75	97	24.25	18.75	1.89	$17.25 < \begin{matrix} 19.14 \\ 15.36 \end{matrix}$
N	400	93	105	158	39.50	26.25	2.12	$23.25 < \begin{matrix} 25.37 \\ 21.13 \end{matrix}$
S	400	97	107	142	35.50	26.75	2.14	$24.25 < \begin{matrix} 26.39 \\ 22.11 \end{matrix}$

Table 11.
Tree 9.
Variation in level of infestation with aspect by Phytomyza ilicis

ASPECT	TOTAL NO. LEAVES	LEAVES WITH MINES	TOTAL NO. OF MINES	TOTAL NO. OF EGGS	EGGS $^{-1}$ 100 LEAVES	MINES $^{-1}$ 100 LEAVES	SE.	INFESTATION + SE.
E	300	41	41	52	17.33	13.66	1.98	$13.66 < {15.64 \atop 11.68}$
W	300	33	33	46	15.33	11.00	1.81	$11.00 < {12.81 \atop 9.19}$
N	300	30	33	47	15.67	11.00	1.73	$10.00 < {11.73 \atop 8.27}$
S	300	32	34	41	13.67	11.33	1.78	$10.67 < {12.45 \atop 8.89}$

Thus a difference in the level of infestation with aspect was observed for most trees investigated, however no constant trend was observed. Generally maximum infestation was observed for East-facing areas.

4.1.4. Height variation.

Variation in the level of infestation with height was tested for Trees 1, 3 and 10. Itwas difficult to sample trees at heights above 5.5 m, besides any results obtained would be unreliable since branches near the crown of tall trees tend to produce fewer leaves per twig.

For Tree 1, samples of 6 twigs per 50 cm height intervals were taken for each side of the tree. (Table 12). As no significant difference in infestation was found with aspect, the values were bulked and expressed as given in Table 13 . A significant difference in total mine number with height was observed. The calculated value for X^2 was 21.95 which is significant at both 5 and 1% levels (Appendix 2). The highest incidence of mines was observed for the height interval, 100 - 150cm, lowest levels of infestation being associated with branches nearest the ground, and at the crown of the tree (Fig5). A significant difference for bulked data was observed again at 5 and 1% significant levels, for egg data (Appendix 2). However although the number of twigs sampled was constant, the total number of leaves varied. Thus although a difference was observed when total mine data was compared with height, when these results were expressed per 100 leaves, little variation was observed.

Since egg distribution was influenced by aspect, tests for significant difference in egg density with both aspect and height were carried out. For the East-facing area of Tree 1, (Appendix 3), a significant difference resulted at 5% level, i.e. calculated X^2 was 15.95 compared with a tabulated value of 9.49. However at the 0 - 50 cm height interval, again leaves were sparse, thus results obtained were erroneous and it is probable that egg-density is independant of height. For other aspects tested significant results were obtained but once more the sample size was inconsistent with regard to the number, hence no conclusions were drawn with regard to significance although from raw data it appears that population density is higher in the 100 - 150 cm interval.

For Tree 3, the problem of varying total leaf number sampled with each height interval was overruled, since a constant sample size of 300 leaves was used (Table 14). X^2 results for both total mine number and egg-density indicate significant results at both 5 and 1% levels (Appendix 3). For total mine data, highest infestation occurred between intervals 0 - 50 and 200 cms, maximum values peaking at 50 - 100 cm interval. From 300 cms upwards the level of infestation tailed off. Similar trends were observed from egg-density data except that levels remained stationary between 150 and 400 cm dropping rapidly from 400cm upwards. For Tree 6, no variation in total mine data or egg-density was observed with height. (Table 15), (Appendix 3).

Tree 1.
Height profile based on a sample of 6 twigs of similar length per 50 cm height interval, and also taking into account variation in infestation with aspect.
Table 12.

ASPECT WITH HEIGHT	TOTAL NO. OF LEAVES	TOTAL NO. OF MINES	TOTAL NO. OF LEAVES WITH MINES	TOTAL NO. OF EGGS	EGGS $^{-1}$ 100 LEAVES	MINES $^{-1}$ 100 LEAVES	SE	INFESTATION WITH SE.
EAST								
0-50	18	6	6	7	38.88	33.30	11.06	$33.30 < \begin{matrix} 44.39 \\ 22.27 \end{matrix}$
50-100	74	13	12	15	20.27	17.37	4.30	$16.20 < \begin{matrix} 20.50 \\ 11.90 \end{matrix}$
100-150	120	21	21	26	21.67	17.50	3.47	$17.50 < \begin{matrix} 20.97 \\ 14.03 \end{matrix}$
150-200	113	13	13	20	17.69	11.50	3.0	$11.50 < \begin{matrix} 14.50 \\ 8.50 \end{matrix}$
200-250	142	24	24	29	20.40	16.90	3.0	$16.90 < \begin{matrix} 19.90 \\ 13.90 \end{matrix}$
WEST								
0-50	253	17	16	23	9.09	6.72	5.89	$6.32 < \begin{matrix} 12.21 \\ 0.43 \end{matrix}$
50-100	151	16	15	16	10.59	9.93	2.29	$9.98 < \begin{matrix} 12.22 \\ 7.64 \end{matrix}$
100-150	133	26	25	33	24.81	19.55	3.40	$18.80 < \begin{matrix} 22.20 \\ 15.40 \end{matrix}$
150-200	328	20	19	25	7.62	6.10	1.29	$5.79 < \begin{matrix} 7.08 \\ 4.50 \end{matrix}$
200-250	169	11	11	11	6.51	6.51	1.89	$6.51 < \begin{matrix} 8.4 \\ 4.62 \end{matrix}$
250-300	81	6	6	9	11.11	7.41	2.90	$7.41 < \begin{matrix} 10.11 \\ 4.51 \end{matrix}$

Tree 1.
Height profile based on a sample of 6 twigs of similar length per 50 cm height interval, and also taking into account variation in infestation with aspect.
Table 12.

ASPECT WITH HEIGHT	TOTAL NO. OF LEAVES	TOTAL NO. OF MINES	TOTAL NO. OF LEAVES WITH MINES	TOTAL NO. OF EGGS	EGGS m^{-1} 100 LEAVES	MINES m^{-1} 100 LEAVES	SE.	INFESTATION WITH SE.
NORTH 0-50	110	9	16	21	19.09	17.27	3.06	$14.55 < \begin{matrix}17.61\\11.49\end{matrix}$
50-100	99	3	2	3	3.03	3.03	1.4	$2.02 < \begin{matrix}3.42\\0.62\end{matrix}$
100-150	137	3	3	5	3.65	2.19	1.25	$2.19 < \begin{matrix}3.44\\0.94\end{matrix}$
150-200	160	18	17	18	11.25	11.25	2.4	$10.63 < \begin{matrix}13.03\\8.23\end{matrix}$
200-250	161	17	17	20	12.24	10.56	2.42	$10.65 < \begin{matrix}12.98\\8.14\end{matrix}$
SOUTH 0-50	129	9	9	9	6.97	6.97	2.24	$6.97 < \begin{matrix}9.21\\4.73\end{matrix}$
50-100	134	25	23	30	22.40	18.66	3.26	$17.16 < \begin{matrix}20.42\\13.90\end{matrix}$
100-150	103	25	23	40	38.83	24.27	4.1	$22.33 < \begin{matrix}26.43\\18.23\end{matrix}$
150-200	153	14	13	17	11.00	9.15	2.79	$8.50 < \begin{matrix}11.27\\5.70\end{matrix}$
200-250	159	11	10	13	8.18	6.92	1.92	$6.30 < \begin{matrix}8.22\\4.48\end{matrix}$
250-300	179	23	21	28	15.64	12.84	2.4	$11.73 < \begin{matrix}14.13\\9.33\end{matrix}$

Table 13.
Bulked height profile data for Tree 1.

HEIGHT BULKED (cms)	TOTAL NO. OF LEAVES	TOTAL NO. OF MINES	TOTAL NO. OF LEAVES WITH MINES	TOTAL NO. OF EGGS	EGGS $^{-1}$ 100 LEAVES	MINES $^{-1}$ 100 LEAVES	INFESTATION WITH SE.	SE.
0.50	510	51	47	60	11.76	10.00	$9.22 < {10.50 \atop 7.94}$	1.28
50.100	458	57	52	64	13.97	12.45	$11.35 < {12.83 \atop 9.87}$	1.48
100.150	498	75	72	103	20.68	15.06	$14.46 < {16.04 \atop 12.82}$	1.58
150.200	754	65	62	80	10.61	8.62	$8.22 < {9.12 \atop 7.30}$	0.9
200.250	631	63	62	73	11.57	9.98	$9.82 < {11.02 \atop 8.62}$	1.2
250.300	260	29	27	37	14.43	11.15	$10.38 < {12.28 \atop 8.48}$	1.9

FIG.5
Variation in total egg/mine numbers with height for Tree 1.

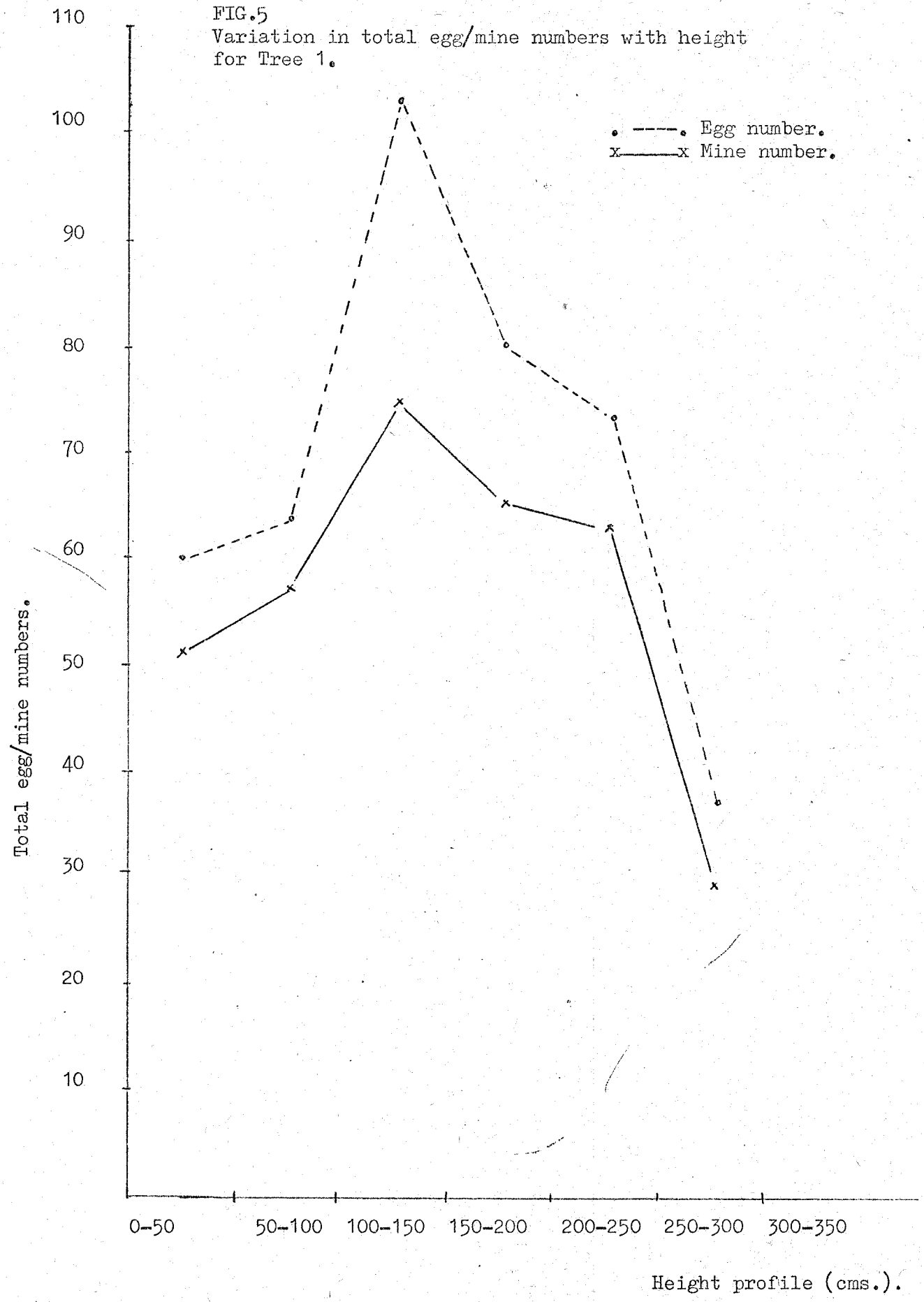


Table 14
Height profile for Tree 3.

HEIGHT (cms)	TOTAL NO. OF LEAVES	TOTAL NO. OF LEAVES	TOTAL NO. OF MINES	TOTAL NO. OF EGGS	MINES -1 100 LEAVES	EGGS -1 100 LEAVES	SE	INFESTATION + SE
0-50	300	66	71	97	23.67	32.33	2.54	$22.00 \lessgtr \begin{matrix} 24.54 \\ 19.46 \end{matrix}$
50-100	300	79	86	131	28.67	43.66	2.54	$26.33 \lessgtr \begin{matrix} 28.87 \\ 24.21 \end{matrix}$
100-150	300	71	76	104	25.33	34.66	2.45	$23.66 \lessgtr \begin{matrix} 26.11 \\ 21.21 \end{matrix}$
150-200	300	65	67	84	22.33	28.00	2.38	$21.67 \lessgtr \begin{matrix} 24.05 \\ 19.29 \end{matrix}$
200-250	300	58	59	78	19.67	26.00	2.28	$19.33 \lessgtr \begin{matrix} 21.61 \\ 17.05 \end{matrix}$
250-300	300	57	61	79	20.30	26.33	2.26	$19.00 \lessgtr \begin{matrix} 21.26 \\ 16.74 \end{matrix}$
300-350	300	48	52	68	17.33	22.66	1.90	$16.00 \lessgtr \begin{matrix} 17.90 \\ 14.10 \end{matrix}$
350-400	300	34	35	69	11.67	23.00	1.83	$11.33 \lessgtr \begin{matrix} 13.16 \\ 9.50 \end{matrix}$
400-450	300	28	28	35	9.33	11.67	1.67	$9.33 \lessgtr \begin{matrix} 11.00 \\ 7.66 \end{matrix}$

Table 15.
Variation in level of infestation with height for Tree 6.
Sampled to 200 cm level only because leaves above this level very sparse hence estimates inaccurate

HEIGHT (cms)	TOTAL NO. OF LEAVES	TOTAL NO. OF MINES	TOTAL NO. OF LEAVES WITH MINES	TOTAL NO. OF EGGS	MINES $^{-1}$ 100 LEAVES	EGGS $^{-1}$ 100 LEAVES	SE.	INFESTATION + SE.
0-50	100	32	25	40	32.00	40.00	4.33	29.33-21.67
50-100	100	26	26	34	26.00	34.00	4.39	30.39-21.61
100-200	100	33	29	44	33.00	44.00	4.14	26.14-18.86
150-200	100	33	29	44	33.00	44.00	4.54	33.54-24.46

Table 16a.
Initial sampling for height profiles with tree 10.

TOTAL NO. OF LEAVES	NO. OF LEAVES WITH MINES	TOTAL NO. OF MINES	TOTAL NO. OF EGGS	MINES $^{-1}$ 100 LEAVES	EGGS $^{-1}$ 100 LEAVES	INFESTATION	SE.	INFESTATION + SE.	HEIGHT
100	32	35	56	35.0	56.00	32.00	4.67	36.57-27.33	LOW
100	26	31	46	31.00	46.00	26.00	4.39	30.39-21.61	MIDDLE
100	12	13	15	13.00	15.00	12.00	3.2	15.2-8.8.	HIGH

Table 16b.
Height profile data for Tree 10.

HEIGHT (cms)	TOTAL NO. OF LEAVES	NO. OF LEAVES WITH MINES	TOTAL NO. OF MINES	TOTAL NO. NO. OF EGGS	MINES 100 $^{-1}$ LEAVES	EGGS 100 $^{-1}$ LEAVES	SE.	INFESTATION + SE.
0-50	300	73	79	119	26.33	39.67	2.47	24.33 $\lessgtr^{26.80}_{21.86}$
50-100	300	89	98	124	32.66	41.33	2.64	29.66 $\lessgtr^{32.30}_{27.02}$
100-150	300	66	78	100	26.00	33.00	2.39	22.00 $\lessgtr^{24.39}_{19.61}$
150-200	300	68	83	122	27.67	40.67	2.43	22.66 $\lessgtr^{25.08}_{20.24}$
200-250	300	61	76	104	25.33	34.47	2.30	20.33 $\lessgtr^{22.63}_{18.03}$
250-300	300	37	39	54	13.00	18.00	1.9	12.33 $\lessgtr^{14.20}_{10.40}$
300-350	300	45	50	63	16.67	21.00	2.06	15.00 $\lessgtr^{17.06}_{12.94}$
350-400	300	33	36	45	12.00	15.00	1.81	11.00 $\lessgtr^{12.81}_{9.19}$

Tree 10 was very large, three main stems arising from a single rhizome, thus an initial sample was taken (Table 16a). A significant difference was observed (Appendix 3) at 1% level using total mine data, lowest levels of infestation being recorded for extreme heights. Results for the main samples are given in Table 16b. Results for egg-density and total mine number were very significant at 1% level, i.e. for mine density the calculated value for X^2 was 53.37 compared with a tabulated value of 18.48 and that for egg-density was 80.45 compared with 18.48 for tabulated values (Appendix 3). Highest values for both egg and mine data were found at 50 - 100 cms. After 250 cms, values for both parameters were reduced considerably.

In summary, it would appear that the level of infestation for Phytomyza is influenced by height, minimum values being recorded from 300 cms upwards.

4.2. Egg distribution and egg mortality.

The frequency of 2 or more mines per leaf was very low for all trees examined (Table 17). For Trees 1,2,4,7,8,9,11,12,13,16, the maximum mine number was 2. Maximum mine number for the remaining trees was 3, but the incidence of 3 mines per leaf was very low in comparison. Where 3 mines were found generally only 1 of these developed successfully to the pupal stage. Usually the second and third mines were empty or contained shrivelled contents, e.g. for Tree 3, 3 mines were found on one leaf. Two were very small hence the larvae had suffered from early mortality factors. The third developed normally but was attacked by birds. On a few occasions 3 pupae were found within a single leaf, although mines were formed on both sides of the leaf to reduce competition for food. It follows that 2 larvae within a leaf will develop more successfully compared with 3. Even so the number of leaves having 2 mines of which only 1 developed normally, was high. For Tree 11, for a sample of 45 leaves where 1 egg had given rise to 1 mine, the number of empty mines and those containing dead larvae was determined, and was found to be 17.78%. A similar estimate for leaves containing 2 mines gave a value of 23.33%. Thus it would appear that competition between several larvae within the leaf reduces the survival of any one.

For egg-density per leaf, maximum numbers observed were 5 per leaf, although the frequency was low i.e. Trees 3,6,9,10,14 and 15. The frequency of viable eggs, i.e. those developing to form visible mines tails off as the number of eggs carried by each leaf increases. Where 5 eggs per leaf were laid, the maximum number surviving to the larval stage was 2. The distribution of Phytomyza eggs for all trees was examined and in every case was found to be clumped (Table 18). Tree 15 had the largest number of leaves with 5 eggs per leaf.

FIG. 6.

Variation in total egg/mine number for Tree 3 with height.

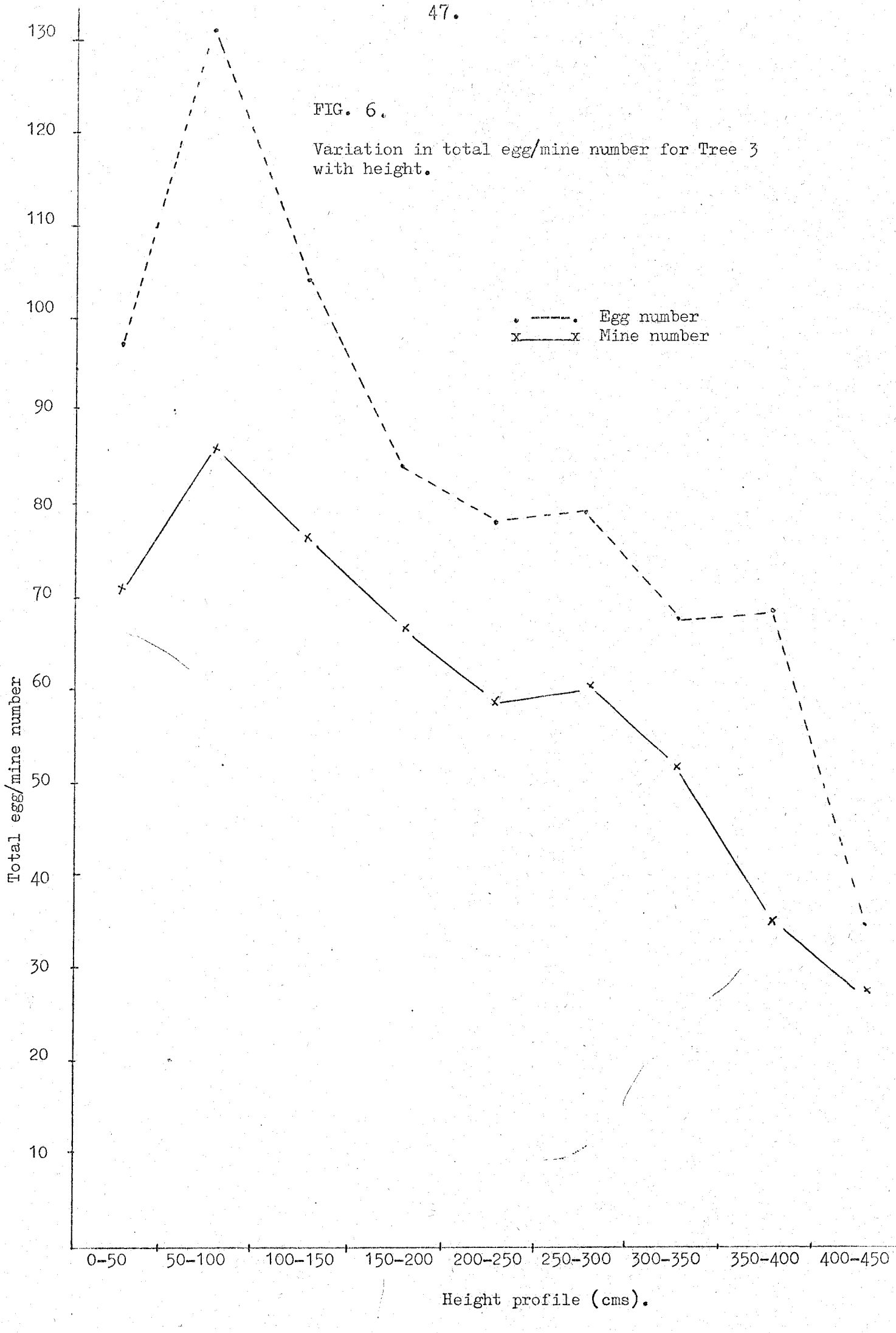


FIG. 7.

Variation in total egg/mine number with height for Tree 10.

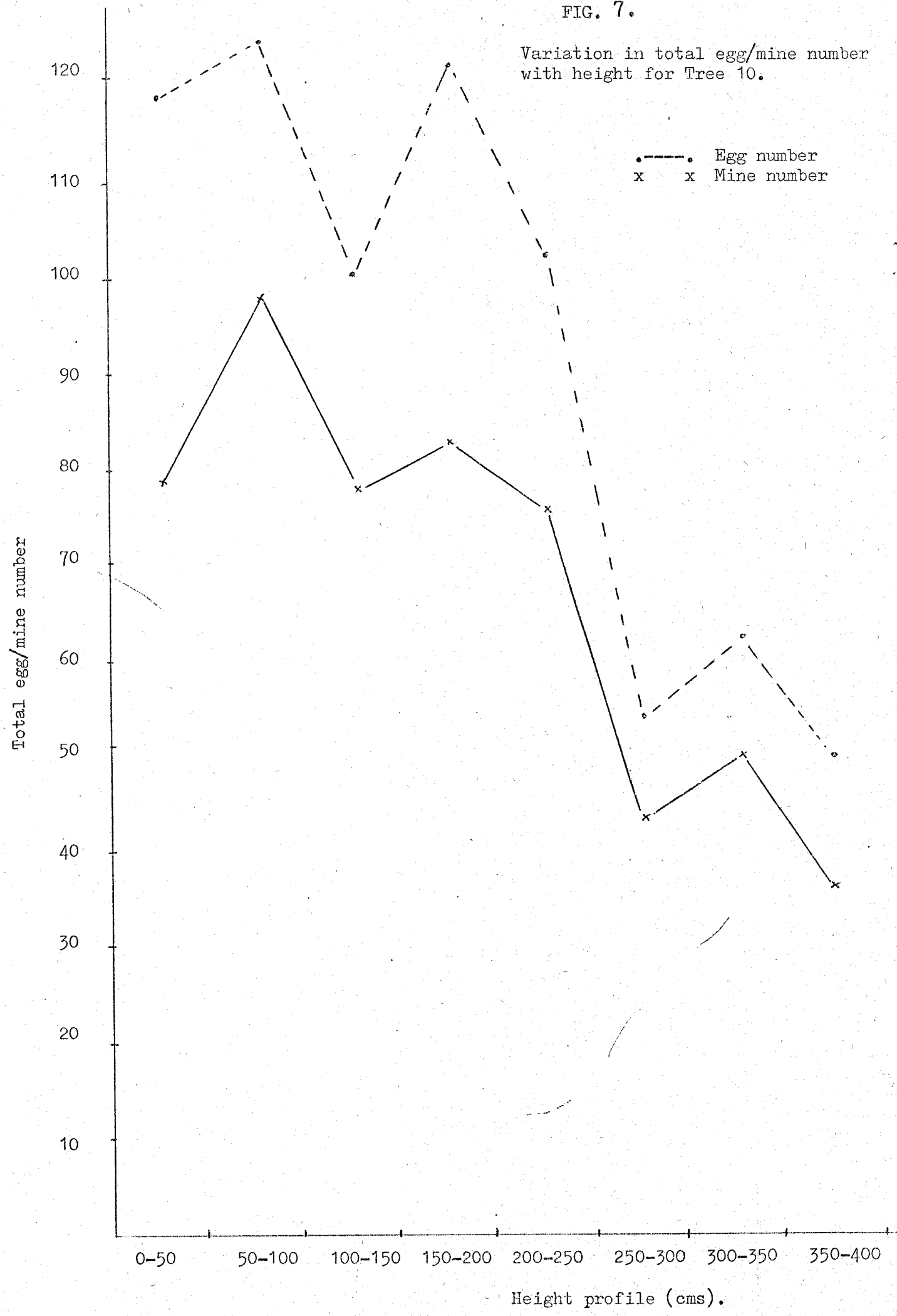


FIG. 8
Summary of variation in mine/egg density expressed per 100 leaves, and total infestation rates for sample trees 1 - 16. (based on total data).

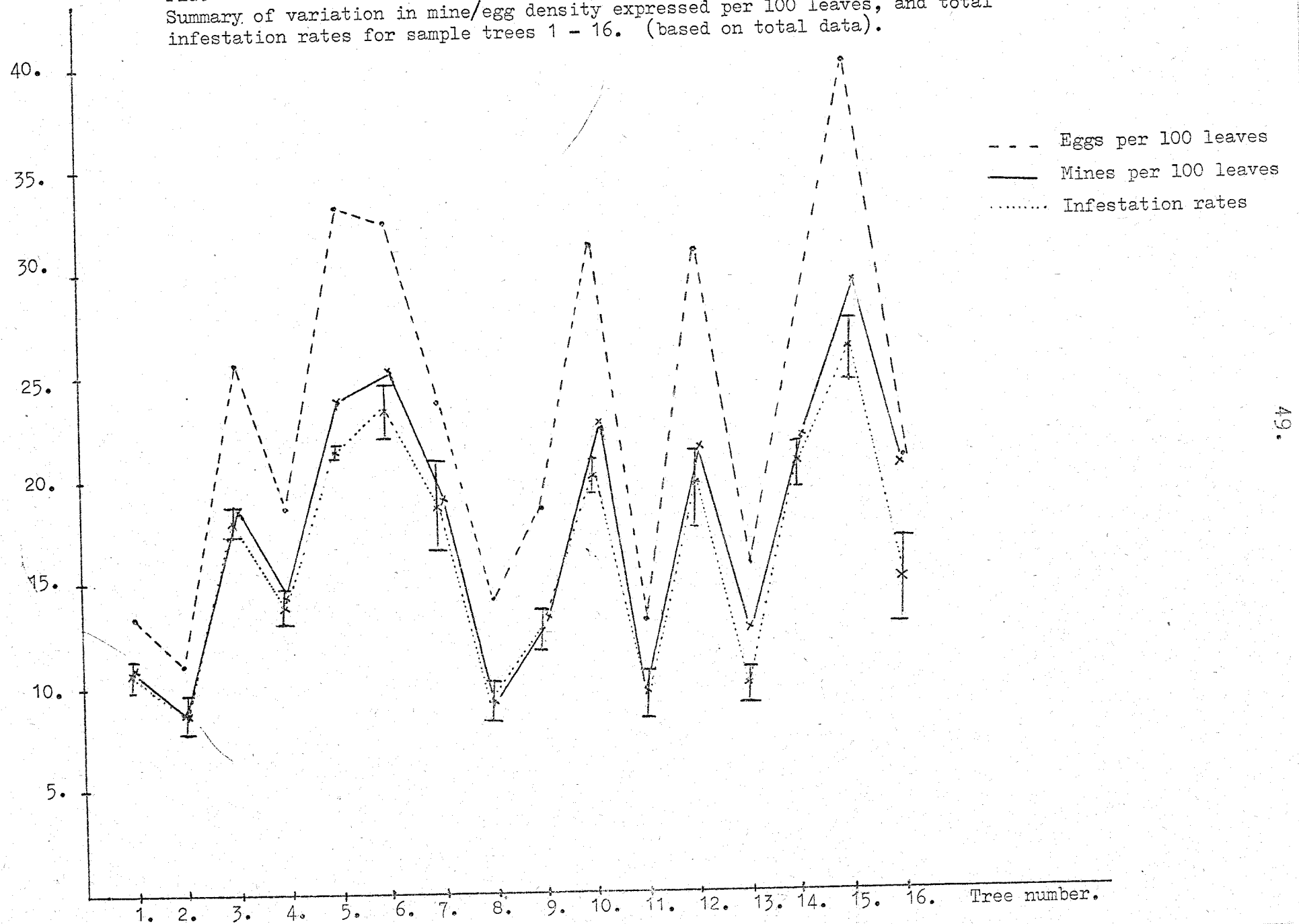


Table 17.

(Tree 1.) Distribution of P. ilicis eggs over Ilex Aquafolium

Frequency				
Eggs	Mines	1	2	3
1.	-	248	-	-
2.	-	48	13	-
3.	-	11	2	-
4.	-	-	2	-
5.	-	-	--	-

(Tree 2.)

Frequency				
Eggs	Mines	1	2	3
1.	-	93	-	-
2.	-	19	3	-
3.	-	3	1	-
4.	-	-	-	-
5.	-	-	-	-

(Tree 3.)

Frequency				
Eggs	Mines	1	2	3
1.	-	536	-	-
2.	-	170	24	-
3.	-	34	7	-
4.	-	8	3	1
5.	-	-	2	-

Table ..17.

(Tree 4)

Frequency

Eggs	Mines	1	2	3
1.	-	155	-	-
2.	-	30	9	-
3.	-	10	2	-
4.	-	1	1	-
5.	-	-	-	-

(Tree 5.)

Frequency

Eggs	Mines	1	2	3
1.	-	230	-	-
2.	-	72	22	-
3.	-	21	8	0
4.	-	7	4	2
5.	-	3	-	-

(Tree 6.)

Frequency

Eggs	Mines	1	2	3
1.	-	167	-	-
2.	-	49	14	-
3.	-	8	4	1
4.	-	2	-	-
5.	-	-	1	-

Table ..17..

(Tree 7.)

Frequency

Eggs	Mines	1	2	3
1.	-	48	-	-
2.	-	9	1	-
3.	-	3	-	-
4.	-	-	-	-
5.	-	-	-	-

(Tree 8.)

Frequency

Eggs	Mines	1	2	3
1.	-	45	-	-
2.	-	17	2	-
3.	-	-	-	-
4.	-	1	-	-
5.	-	-	-	-

(Tree 9.)

Frequency

Eggs	Mines	1	2	3
1.	-	121	-	-
2.	-	36	4	-
3.	-	7	2	-
4.	-	4	2	-
5.	-	1	1	-

Table .17..

(Tree 10.)

Frequency

Eggs	Mines	1	2	3
1.	-	340	-	-
2.	-	94	33	-
3.	-	32	20	5
4.	-	10	6	2
5.	-	1	1	-

(Tree 11.)

Frequency

Eggs	Mines	1	2	3
1.	-	56	-	-
2.	-	11	3	-
3.	-	1	-	-
4.	-	-	-	-
5.	-	-	-	-

(Tree 12.)

Frequency

Eggs	Mines	1	2	3
1.	1	101	-	-
2.	-	37	9	-
3.	1	13	6	-
4.	-	2	-	-
5.	-	-	-	-

Table ..17..

(Tree 13.)

Frequency

Eggs	Mines	1	2	3
1.	-	61	-	-
2.	-	14	5	-
3.	-	1	1	-
4.	-	2	-	-
5.	-	-	-	-

(Tree 14.)

Frequency

Eggs	Mines	1	2	3
1.	-	169	-	-
2.	-	35	8	-
3.	-	10	4	-
4.	-	1	1	1
5.	-	1	-	-

(Tree 15.)

Frequency

Eggs	Mines	1	2	3
1.	-	129	-	-
2.	-	38	10	-
3.	-	10	8	1
4.	-	2	2	1
5.	-	2	-	-

Table .17..

(Tree 16.)

Frequency

Eggs	Mines	1	2	3
1.	-	45	-	-
2.	-	7	5	-
3.	-	2	-	-
4.	-	1	3	-
5.	-	-	-	-

Table ..18.

Distribution of P. ilicis eggs for all Trees Examined

Egg Number Per Leaf						$\bar{x}$	S^2	$S^2 : \bar{x}$	Distribution	
0	1	2	3	4	5					
2786	248	61	13	2	0	0.134	0.188	$S^2 > x$	CLUMPED	Tree 1.
1263	93	22	4	0	0	0.108	0.145	"	"	Tree 2.
3195	536	194	41	12	2	0.257	0.382	"	"	Tree 3.
1214	155	39	12	2	0	0.186	0.268	"	"	Tree 4.
1146	230	94	29	13	3	0.403	1.93	"	"	Tree 5.
830	167	63	13	2	1	0.32	0.449	"	"	Tree 6.
248	48	10	3	0	0	0.237	0.299	"	"	Tree 7.
604	45	19	4	1	0	0.141	0.227	"	"	Tree 8.
1138	121	40	14	8	2	0.187	0.383	"	"	Tree 9.
2083	340	127	57	18	2	0.314	0.531	"	"	Tree 10.
586	56	28	-	4	-	0.131	0.173	"	"	Tree 11.
585	102	92	60	8	-	0.309	0.493	"	"	Tree 12.
601	61	38	6	8	-	0.158	0.237	"	"	Tree 13.
799	169	86	42	12	5	0.208	1.04	"	"	Tree 14.
464	129	96	57	20	10	0.402	0.641	"	"	Tree 15.
311	45	24	6	16	0	0.226	0.385	"	"	Tree 16.

FREQUENCY VALUES

The percentage mortality of eggs for each tree, i.e. (total number of eggs) - (total number of viable mines produced), divided by total egg number was determined (Table 19a). Additional information for egg mortality is given in Table 19b. From Table 19a generally the higher the total number of eggs, the higher the percentage failure rate for egg development to the larval stage. For Table 19b using information for the entire tree, this trend was not so apparent. Maximum egg mortality was observed for Trees 3,5,8,9,10,12, and 15. Minimum egg mortality was observed for Trees 1,2 and 16.

Fig. 9 indicates total egg number plotted against total viable mines produced. From regression analysis, values for the straight line equation, $y = mx+c$ were given as $y = 0.73x + 4.86$, correlation coefficient (r) of 0.9985. When egg mortality per 100 leaves was plotted against egg density per 100 leaves, a straight line graph $y = 0.0027x - 0.0081$ was found, and r equalled 0.947 (Fig.10).

From Fig. 11 percentage egg mortality versus egg number, an r value of 0.31 was observed, which is significant at 5% level of significance only. The values for the straight line equation were $y = 0.0098x + 20.17$. For a density-dependant relationship to operate, an increase in egg-density should result in a proportional increase in egg mortality. Although this was outlined to some degree in Fig.11, the fluctuation around mortality values for low levels of egg numbers is too great. When the frequency data for egg numbers per leaf Table 17, was expressed as % values for total egg density per tree, trees expected to have higher % mortality assuming reduced survival of eggs with increasing number borne per leaf, were Trees 3,5,8,9,10,12, and 15. Those with highest survival were for Trees 1,2,4,6,7,13,14, and 16, (Table 19a) These results were confirmed in Table 20. Thus it appears that egg-density per leaf is important in determining the number of eggs which survived per leaf, hence total viable number for the entire tree. However the relationship between egg mortality and egg-density was assumed to be density - independant.

4.3. Major sources of larval and pupal mortality for P. ilicis.

Egg mortality was observed to range from 8.79% - 32.32% (Table 19a). Factors influencing survival of both pupae and larvae within the mine were investigated, and recorded as in Table 21. Each factor was expressed as a % value of the total number of mines recorded for each tree as given. From the point of view of mine mortality major factors influencing the survival of P. ilicis was attack by birds and infestation by the larval parasite, Chrysocharis gemma. A large proportion of mines examined were found to be empty, and these were generally of small diameter, (compare 0.5x0.75 cm with 3.0x1.38 cm for a mine containing P. ilicis pupae). Obviously these eggs had developed to the early larval stage, but reasons for larval mortality were undetermined. Where more than 1 mine was found per leaf it is likely that some factor is operating to ensure the survival of 1 larva at the expence of others, however this does not explain why empty mines were recorded for leaves with only 1 mine.

Table ..19a

Percentage Mortality of eggs from sampled Trees

Tree No.	Total No. of Eggs Laid	No. Surviving to produce mines	% Mortality of Eggs
Tree 1.	417	340	18.47
Tree 2.	149	124	16.78
Tree 3.	1105	804	27.24
Tree 4.	277	219	20.93
Tree 5.	571	411	28.02
Tree 6.	345	272	21.16
Tree 7.	77	62	20.83
Tree 8.	99	67	32.32
Tree 9.	262	286	29.01
Tree 10.	847	617	28.15
Tree 11.	88	67	23.86
Tree 12.	262	182	30.53
Tree 13.	113	90	20.35
Tree 14.	314	245	21.97
Tree 15.	312	227	27.24
Tree 16.	91	83	8.79

Table .1.9b

Estimates of Egg failure to produce viable larvae

(Tree 1.)
(Bulked Data)

Aspect	Total No. of Eggs	Total No. of Mines	% Failure of Eggs Larvae
East	98	77	21.43
West	115	96	16.52
North	67	60	10.45
South	137	107	27.01

Height	Total No. of Eggs	Total No. of Mines	% Failure of Eggs Larvae
0-50cms	60	51	15.00
50-100cms	64	57	10.94
100-50cms	103	75	27.18
150-200cms	80	65	18.75
200-250cms	73	63	13.69
200-300cms	37	29	21.62

(Tree 2.)

Aspect	Total No. of Eggs	Total No. of Mines	% Failure of Eggs Larvae
East	65	48	26.15
West	20	18	10.00
North	34	29	14.71
South	30	29	3.33

(Tree 3.)

Aspect	Total No. of Eggs	Total No. of Mines	% Failure of Eggs Larvae
East	58	47	18.97
West	128	94	26.56
North	80	59	26.25
South	94	69	26.60

Height	Total No. of Eggs	Total No. of Mines	% Failure of Eggs Larvae
0-50cms	97	71	26.80
50-100cms	131	86	34.35
100-150cms	104	76	26.92
150-200cms	84	67	20.24
200-250cms	78	59	24.36
250-300cms	79	61	22.78
300-350cms	68	52	23.54
350-400cms	69	35	49.28
400-450cms	35	28	20.00

(Tree 4.)

Aspect	Total No. of Eggs	Total No. of Mines	% Failure of Eggs Larvae
East	51	39	23.53
West	103	76	26.21
North	57	46	19.30
South	47	40	14.89

(Tree 5.)

Aspect	Total No. of Eggs	Total No. of Mines	% Failure of Eggs Larvae
	130	89	31.54
	97	75	22.68
	158	105	33.54
	142	107	24.65

(Tree 9.)

Aspect	Total No. of Eggs	Total No. of Mines	% Failure of Eggs Larvae
East	52	41	21.15
West	46	33	28.26
North	47	33	29.79
South	41	34	17.07

(Tree 6.)

Height	Total No. of Eggs	Total No. of Mines	% Failure of Eggs Mines
0-50cms	40	32	20.00
50-100cms	34	26	23.53
100-150cms	33	24	27.27
50-200cms	44	33	25.01

(Tree 10.)

Height	Total No. of Eggs	Total No. of Mines	% Failure of Eggs Mines
0-50cms	119	79	33.61
50-100cms	124	98	20.97
100-150cms	100	78	22.01
150-200cms	122	83	31.97
200-250cms	104	76	26.93
250-300cms	54	39	27.78
300-350cms	64	50	20.63
350-400cms	45	36	20.00

Table .....20.

Egg - frequency data for leaves with 1 and 3 eggs per leaf, expressed as a % of total frequency recordings for all trees.

Tree No.	1 egg per leaf	3 eggs per leaf
1	59.14	3.11
2	62.42	2.68
3	48.51	3.71
4	55.96	4.33
5	40.28	5.02
6	61.40	3.77
7	62.34	3.90
8	48.45	4.04
9	46.18	5.34
10	40.14	6.73
11	63.63	0
12	38.98	22.90
13	53.98	5.31
14	53.82	13.37
15	41.35	18.27
16	49.45	6.59

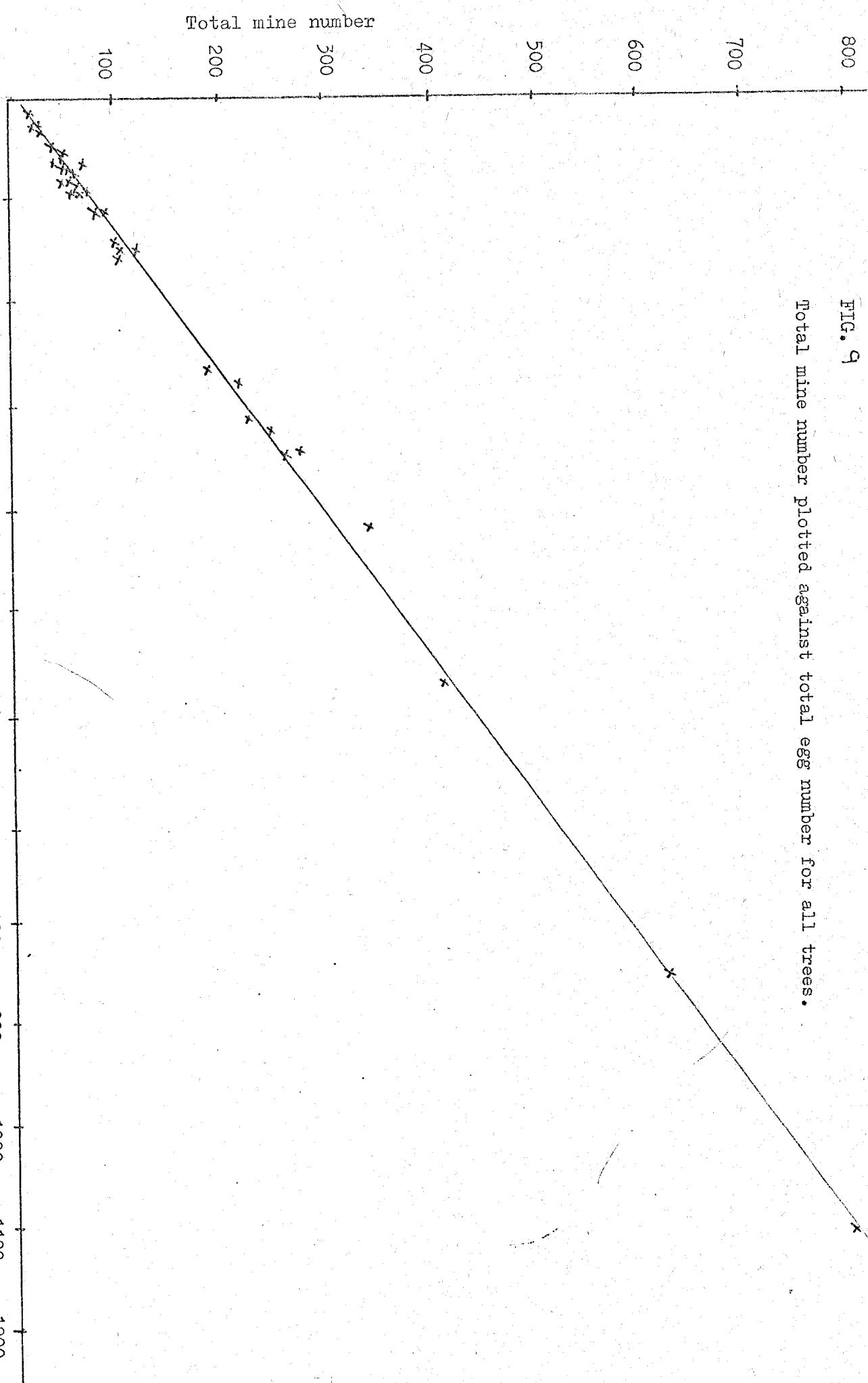


FIG. 9

Total mine number plotted against total egg number for all trees.

Fig.10.

Egg mortality per 100 leaves plotted against egg density per 100 leaves.

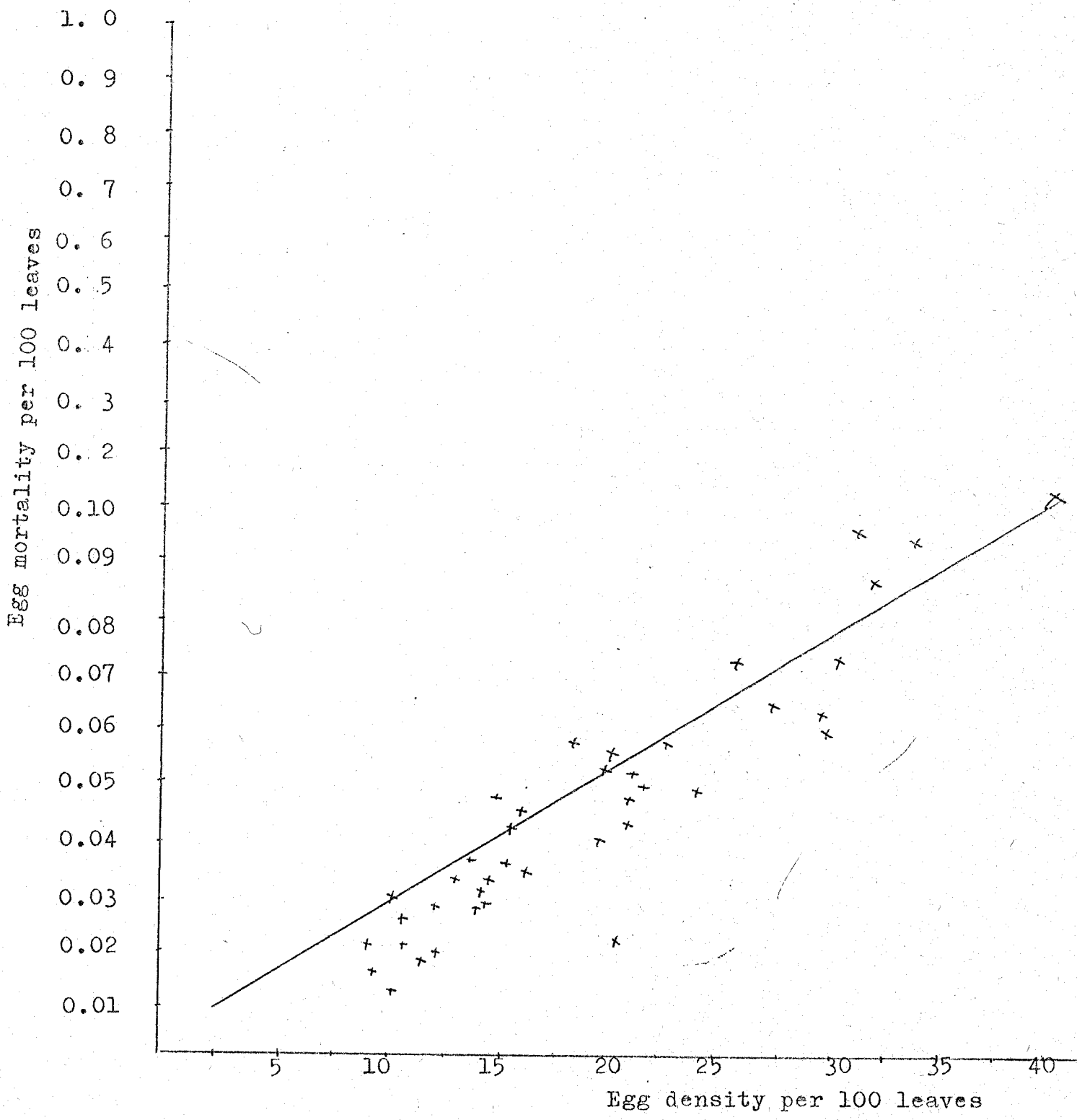
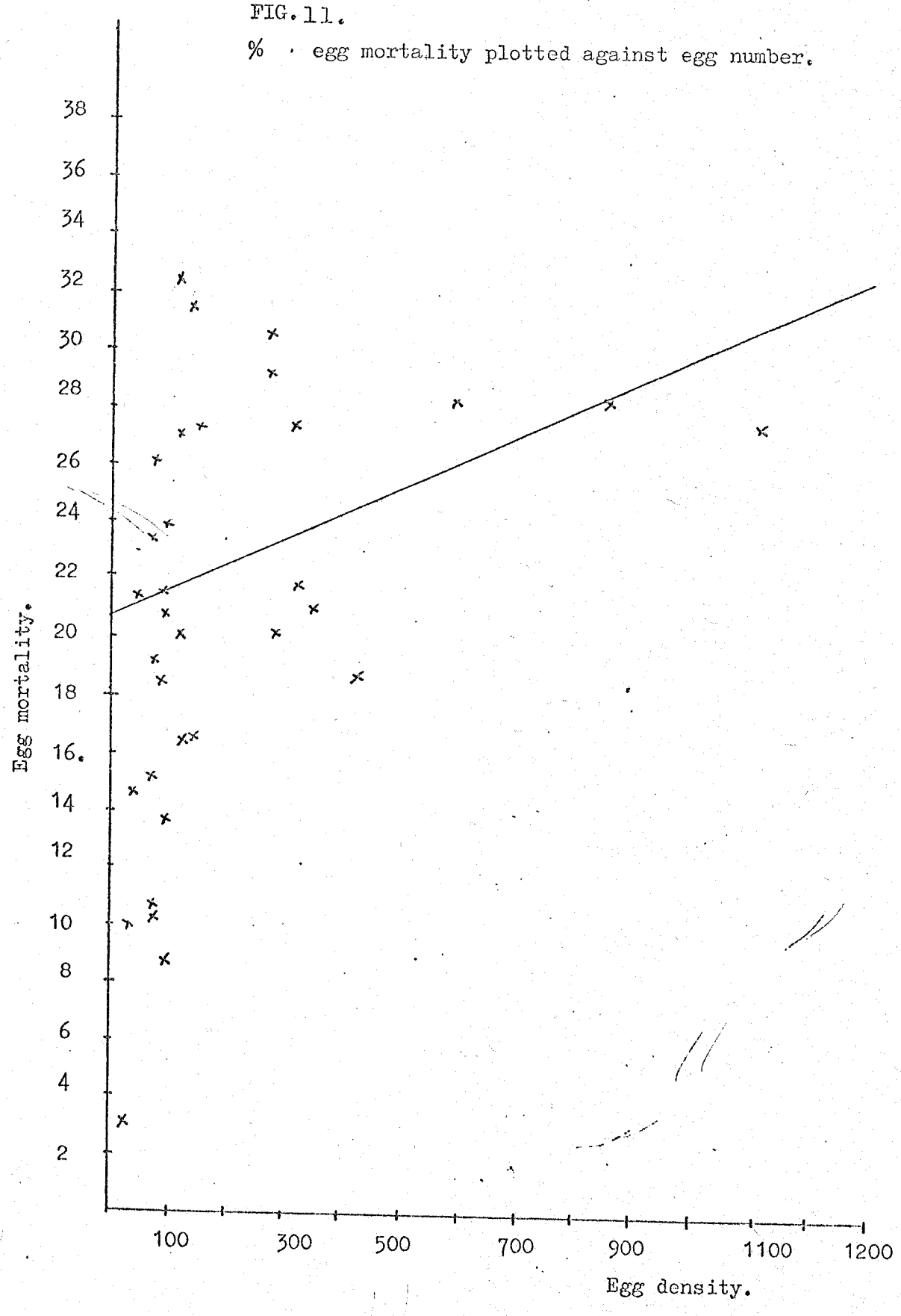


FIG. 11.

% · egg mortality plotted against egg number.



Factors influencing survival of P. ilicis within the leaf are given in Table 21, Figs.12 ,13,14, and15 . With the exception of Trees 6,11,13 and 16, the major cause of mortality within the leaf was attack due to bird-feeding. Attack by birds was most prominent for Trees 2,5,9 and 10, accounting for 70.16, 57.18, 49.46 and 48.62% of total factors respectively,(Fig.13) Trees least affected were 6,7,13,14,15, and 16, where values for bird-pecks were 19.85, 37.10, 24.44, 24.90, 15.86 and 8.44%.

For Tree 6, 37.50% of larvae survived to the pupal stage (Fig.13) Of the remaining factors larval parasitism by Chrysocharis gemma was the most important cause of mortality, and this accounted for 22.06%. Attack by bird-feeding accounted for 19.85%.

For Tree 11, attack on mines by feeding birds, the number of successful larvae surviving to the pupal stage, and the total number of empty mines observed were found to be of equal value when expressed as a percentage of total recordings (Fig.13). They each accounted for 23.88% of total recordings.

For Tree 13, the prominent factor was attack by C. gemma, and this was true for Tree 16. Values observed were 32.22% and 33.73% respectively. Highest larval survival to the pupal stage was observed for Trees 14 and 15, i.e. 57.14 and 33.48%.

From Fig.13 ,14, and 15, the variation in importance of each factor with respect to sample trees is outlined. The number of larvae surviving to pupation was under 40% for all sites examined, except for Tree 14, where survival was exceptional, i.e. 57.14%. Trees 1,6,9,15 and 16, had values of 33.30 - 37.50% for larval - pupae survival. Minimum survival due to the influence of other mortality factors was observed for Trees 2,3,10, and 12, where values between 16.48 - 20.16 were recorded. The incidence of C. gemma attacking larval P. ilicis was recorded in Fig.14. The parasite was not recorded for Trees 1 and 2. Maximum incidence was recorded for Trees 13,15, and 16 i.e. 32.22, 31.28 and 33.73% respectively. Larvae least affected by the parasite were from Trees 3,5 and 14, where values of 8.88, 3.72 and 9.00 were recorded.

In relation to other factors influencing survival of P. ilicis within the mine, the number of empty mines recorded were low. Trees where maximum numbers of empty mines were recorded were 13,15 and 16 (Fig.15), where values of 14.44, 16.74 and 20.48 were observed. Mines least affected were for Trees 9 and 10.

The variation in the number of unhealthy P.ilicis larvae extracted from the mines is given in Fig.15 , this factor being of least importance. The highest values for unhealthy larvae were recorded for Trees 6,7 and 11, i.e. 6.25, 8.06 and 14.93%.

Chi-square tests for significant differences between trees for bird-pecks, attack by C. gemma, the number of empty mines and successful development to pupal stages were tested. It was thought initially that error would be introduced into the results, since all trees were sampled within a given time period, hence exposure time for the last trees to be examined would be greater, especially with regard to bird-attack.

Table 21.

Factors influencing P. ilicis survival after successful egg development (to the pupal stage).

Mortality Factor		Factor expressed as % of total
Bird peck	175	51.47
Chrysocharis gemma-larval Parasite	0	0
Unhealthy P. ilicis larvae	12	3.52
Empty mines	29	8.53
Larvae surviving to pupal stage.	124	36.47

TREE 1.

Mortality Factor		Factor expressed as % of total
Bird peck	87	70.16
Chrysocharis gemma.	0	0
Unhealthy P. ilicis larvae	3	2.42
Empty mines	9	7.26
Larvae surviving to pupal stage.	25	20.16

TREE 2.

Mortality Factor		Values expressed as % of total.
Bird peck	459	57.38
C. gemma larval parasite	71	8.88
Unhealthy P. ilicis larvae	33	4.13
Empty mines	74	9.25
Larvae surviving to pupal stage.	163	20.38

TREE 3.

Mortality Factor		Factor expressed as % of total
Bird peck	70	33.82
C. gemma	47	22.71
Unhealthy P. ilicis lavae	13	6.28
Empty mines	23	11.11
Larvae surviving to pupal stage	54	26.09

TREE 4.

Mortality Factor		Factor expressed as % of total
Bird peck	215	57.18
C. gemma	14	3.72
Unhealthy P. ilicis larvae	23	6.12
Empty mines	29	7.71
Larvae to Pupae	95	25.27

TREE 5.

Mortality Factor		Factor expressed as % of total
Bird peck	54	19.85
C. gemma	60	22.06
Unhealthy P. ilicis larvae	17	6.25
Empty mines	39	14.34
Larvae surviving to pupae	102	37.50

TREE 6.

TREE 7.

Mortality Factor		Factor expressed as % of total
Bird peck	23	37.10
C. gemma	12	19.35
Unhealthy P. ilicis larvae	5	8.06
Empty mines	6	9.68
Larvae surviving to pupal stages	17	27.42

TREE 8.

Mortality Factor		Factor expressed as % of total
Bird peck	30	44.78
C. gemma	10	14.93
Unhealthy P. ilicis	2	2.99
Empty mines	8	11.94
Larvae surviving to pupae	17	25.37

TREE 9.

Mortality Factors		Factors expressed as % of total
Bird peck	92	49.46
C. gemma	20	10.75
Unhealthy P. ilicis	10	5.38
Empty mines	2	1.075
Larvae surviving to pupae	62	33.33

TREE 10.

Mortality Factor		Factors expressed as % of total
Bird peck	300	48.62
C. gemma	96	15.56
Unhealthy P. ilicis larvae	27	4.38
Empty mines	142	23.01
Larva surviving to pupae	122	19.77

TREE 11.

Mortality Factor		Factors expressed as % of total
Bird peck	16	23.88
C. gemma	9	13.43
Unhealthy P. ilicis larvae	10	14.93
Empty mines	16	23.88
Larvae surviving to pupal stage	16	23.88

TREE 12.

Mortality Factor		Factor expressed as % of total
Bird peck	60	32.97
C. gemma	39	21.43
Unhealthy P. ilicis larvae	6	3.29
Empty mines	45	24.73
Larvae to pupal stage	24	26.67

Mortality Factor		Factor expressed as % of total
Bird peck	22	24.44
C. gemma	29	32.22
Unhealthy P. ilicis larvae	2	2.22
Empty mines	13	14.44
Larvae to pupal stage	24	26.67

TREE 13.

Mortality Factor		Factor expressed as % of total
Bird peck	61	24.90
C. gemma	22	9.00
Unhealthy P. ilicis larvae	2	0.80
Empty mines	20	8.16
Larvae surviving to pupal stage	140	57.14

TREE 14.

Mortality Factor		Factor expressed as % of total
Bird peck	36	15.86
C. gemma	71	31.28
Unhealthy P. ilicis larvae	5	2.20
Empty mines	38	16.74
Larvae surviving to pupal stage	76	33.48

TREE 15.

TREE 16.

Mortality Factor		Expressed as % of total
Bird peck	7	8.44
C. gemma	28	33.73
Unhealthy P. ilicis larvae	4	4.82
Empty mines	17	20.48
Larvae surviving to pupal stage.	27	32.53

FIG. 12.
Summary of the factors influencing survival of P. ilicis within the mine to the pupal stage, expressed as a histogram.

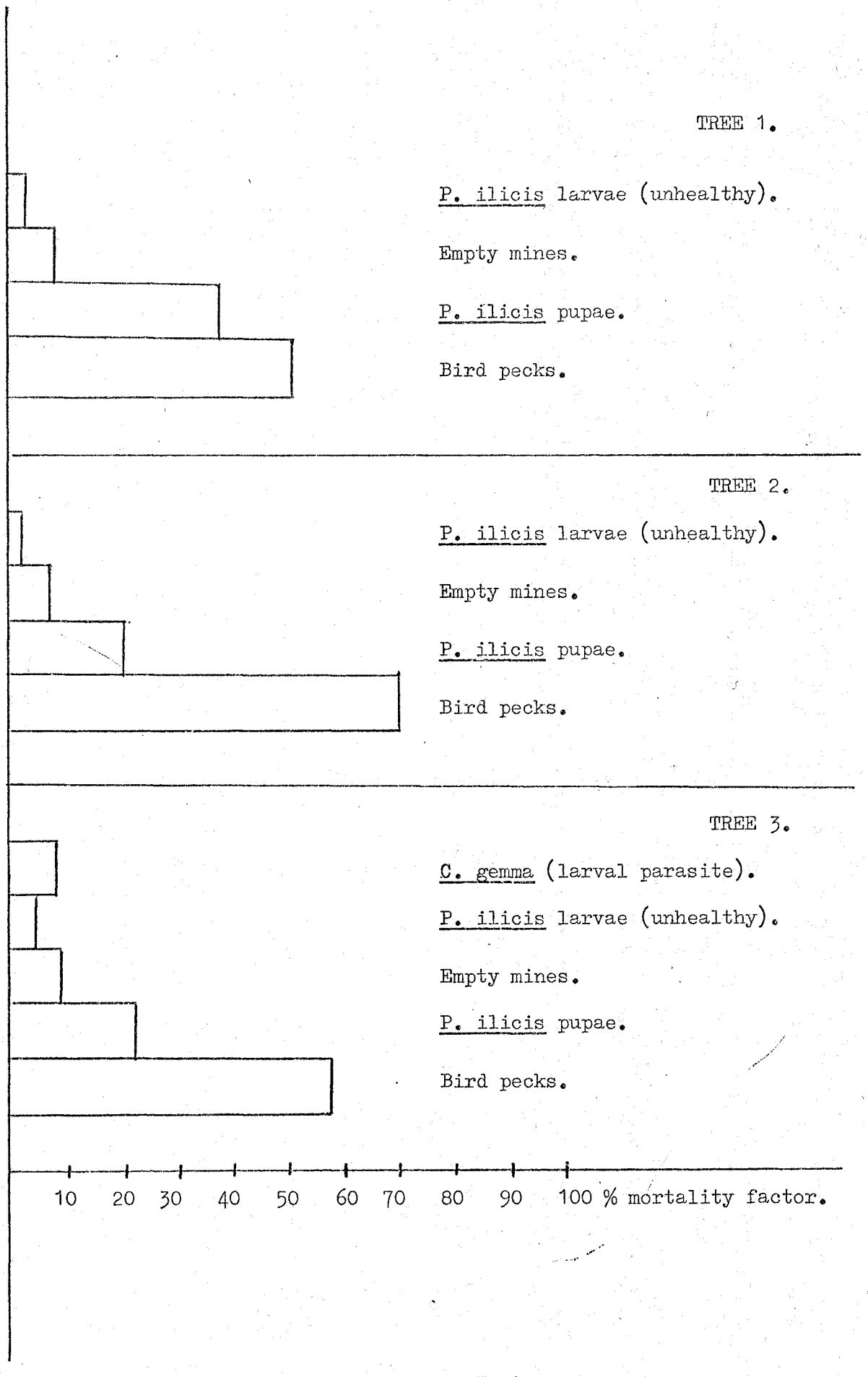


FIG. 12

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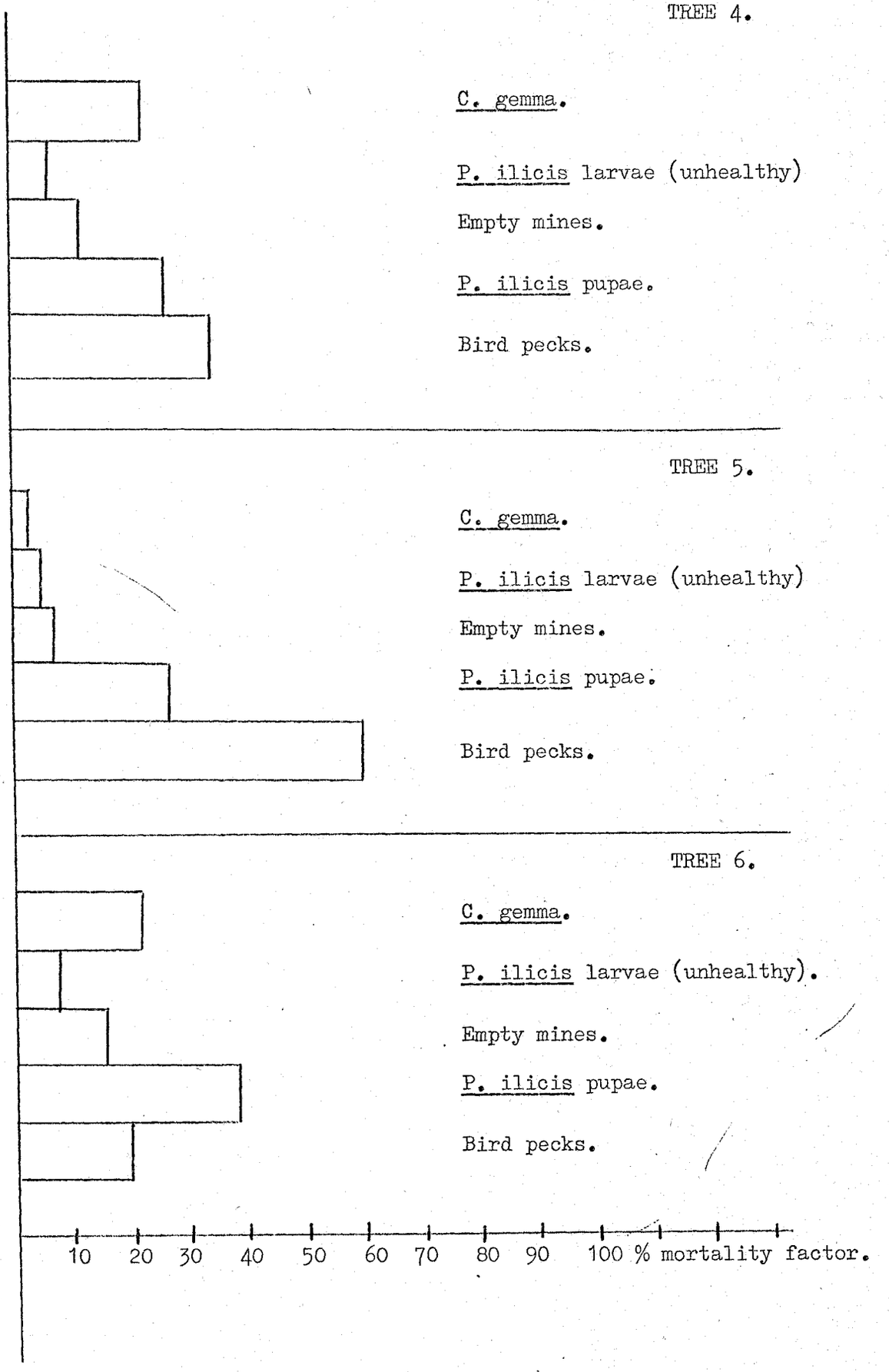

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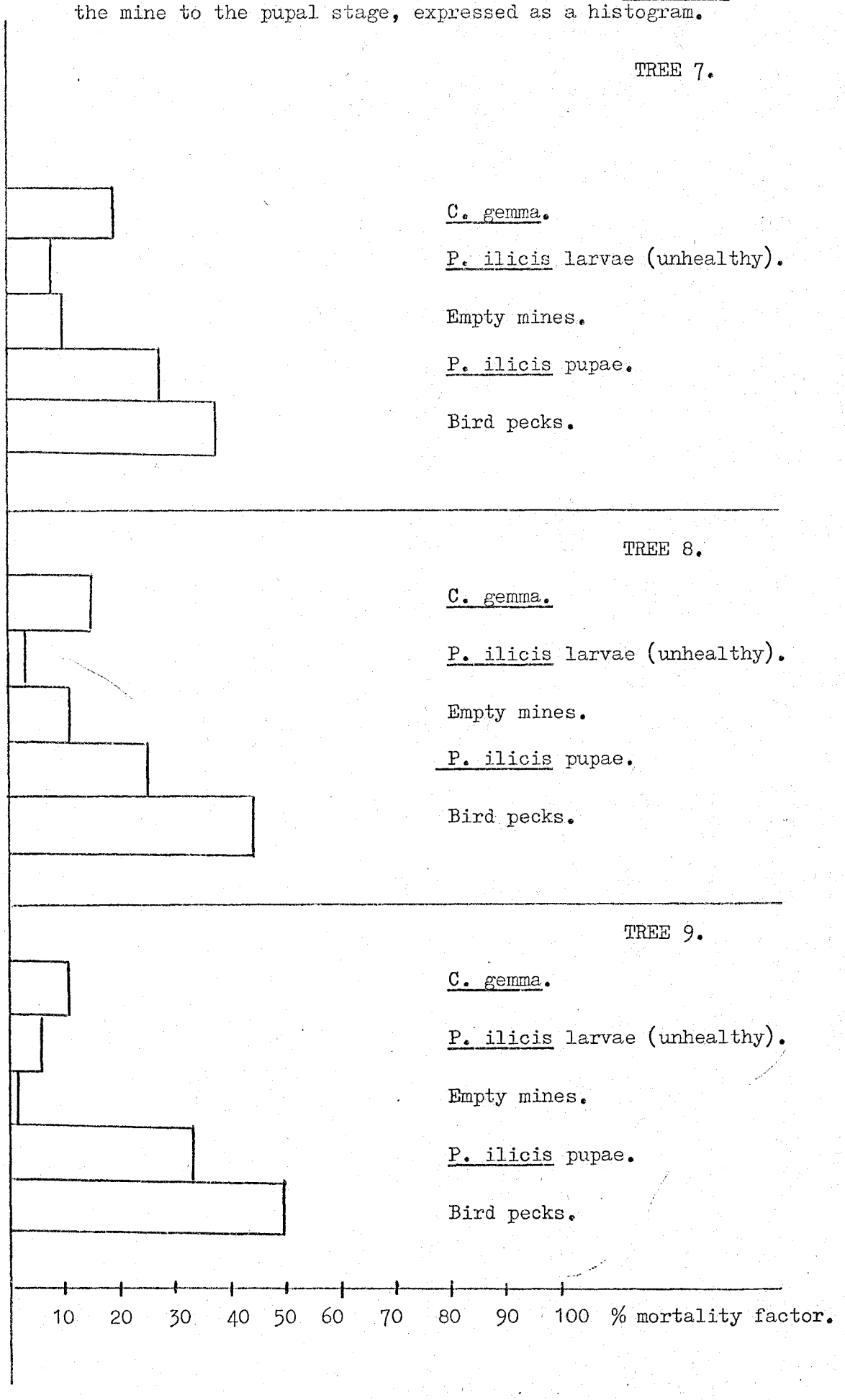

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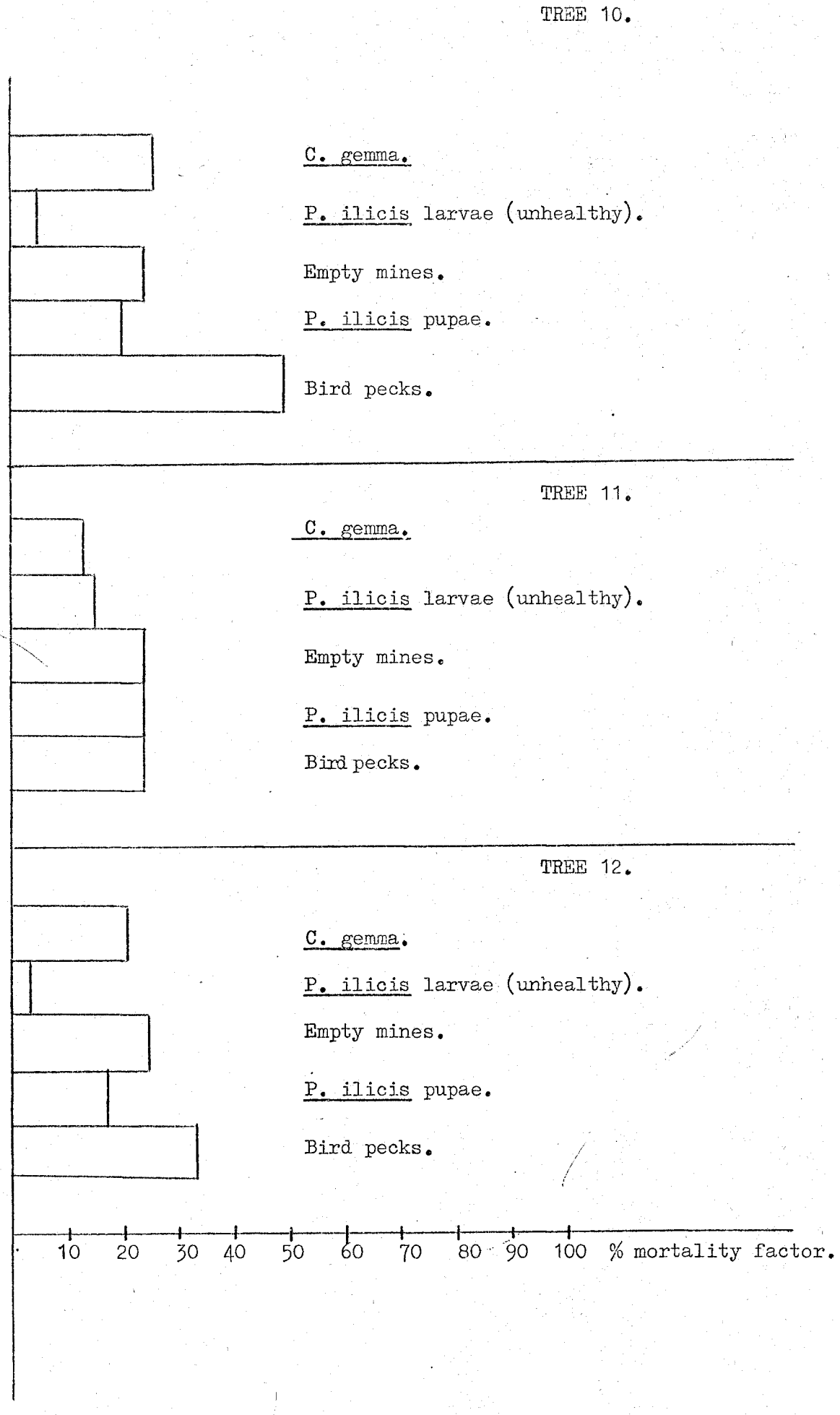

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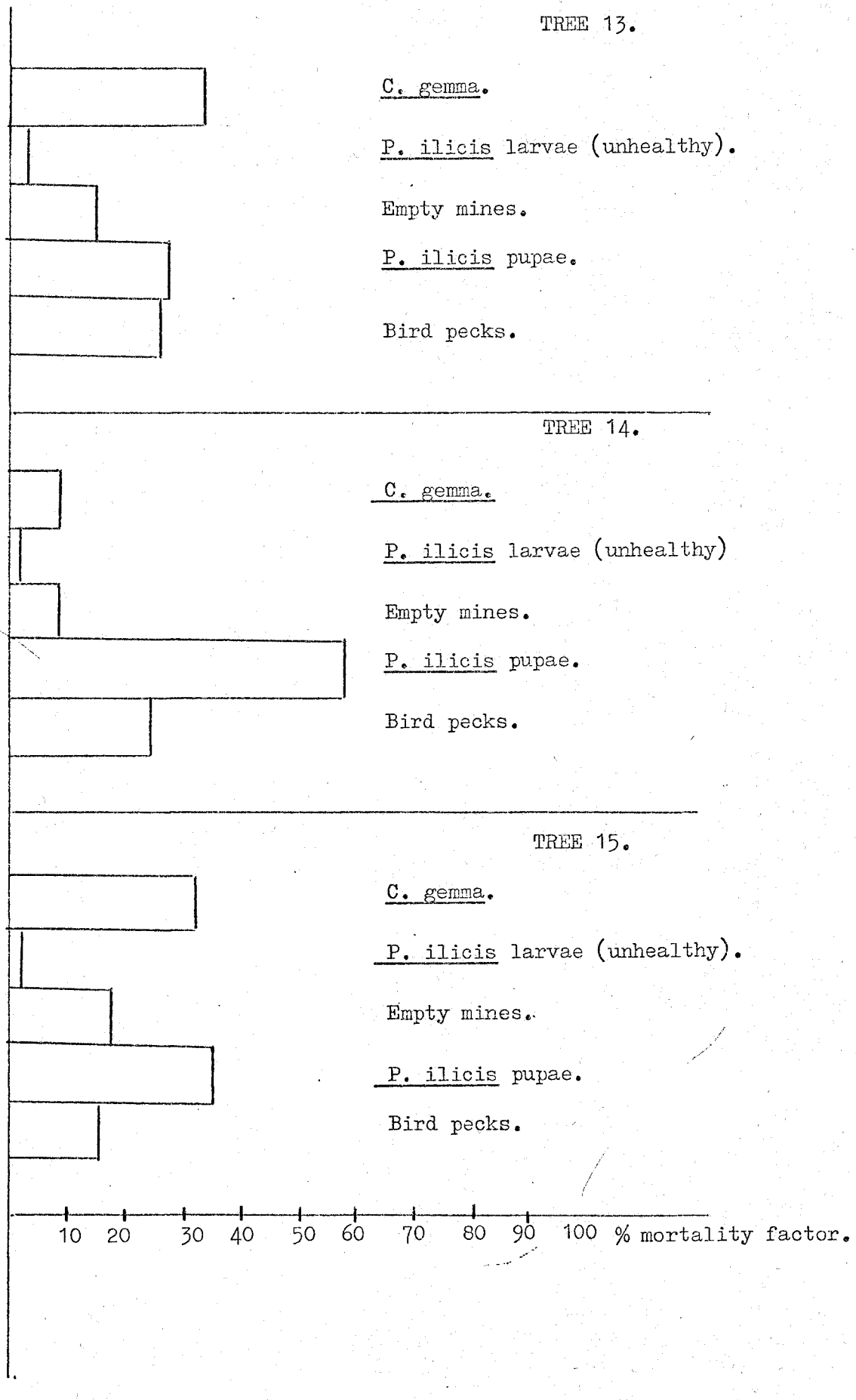

Fig. 12.
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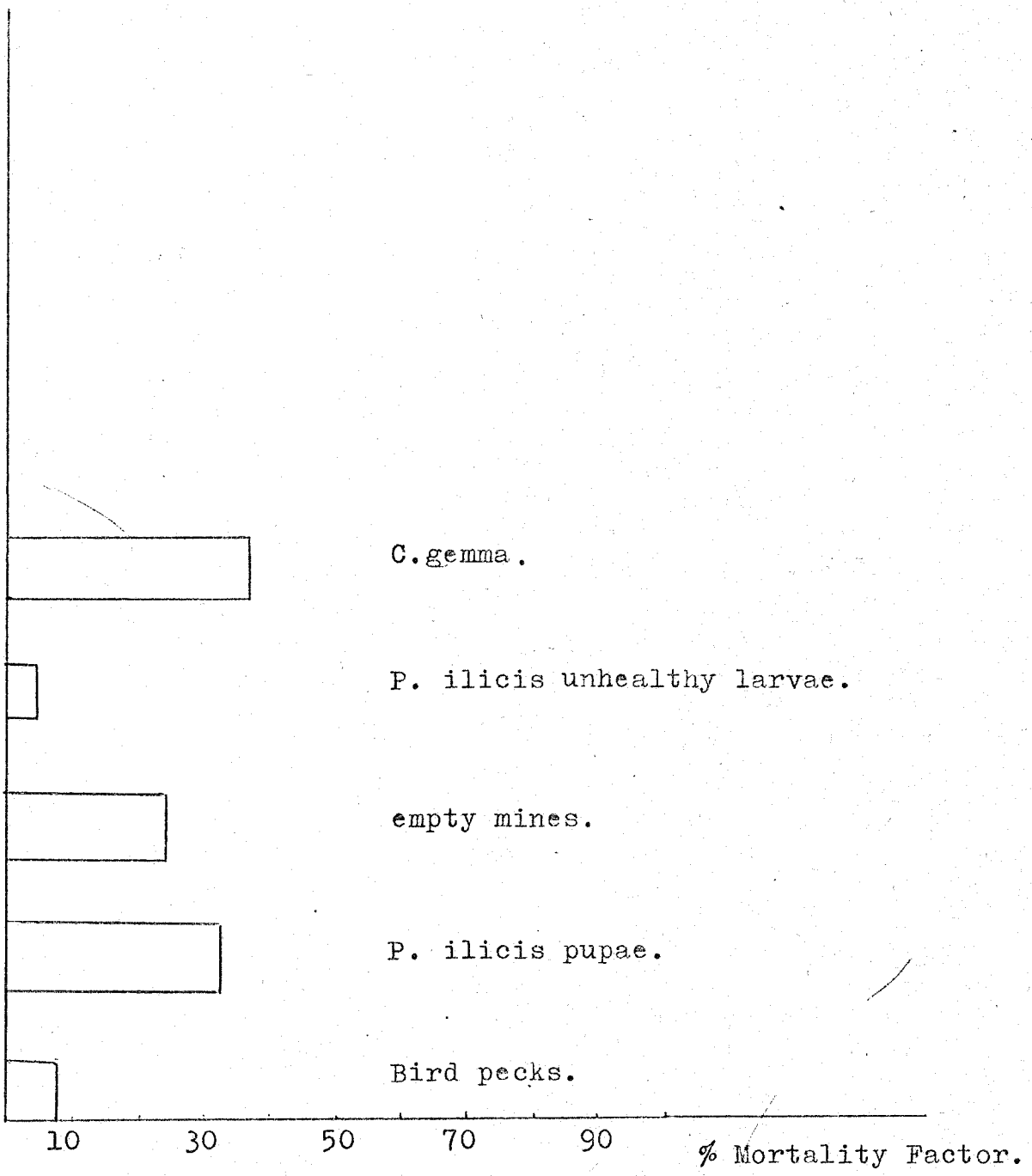


FIG. 13

The variation in bird attack between sample trees together with the number of P. ilicis larvae surviving to the pupal stage, expressed as a % of total factors influencing the survival of P. ilicis in the mine.

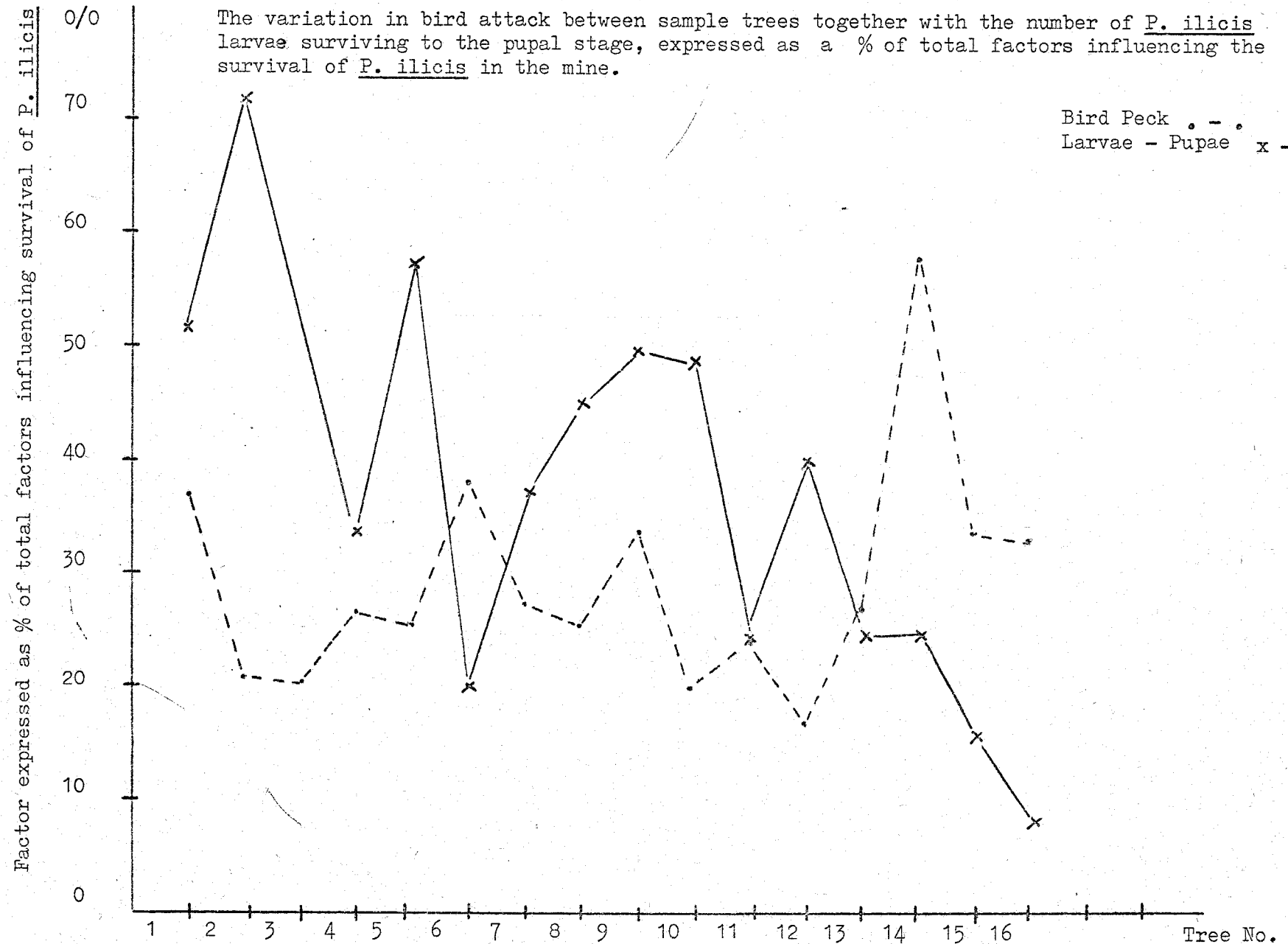


FIG. 14

The variation in the effect of the larval parasite C. gemma on P. ilicis sampled from trees 1 - 16

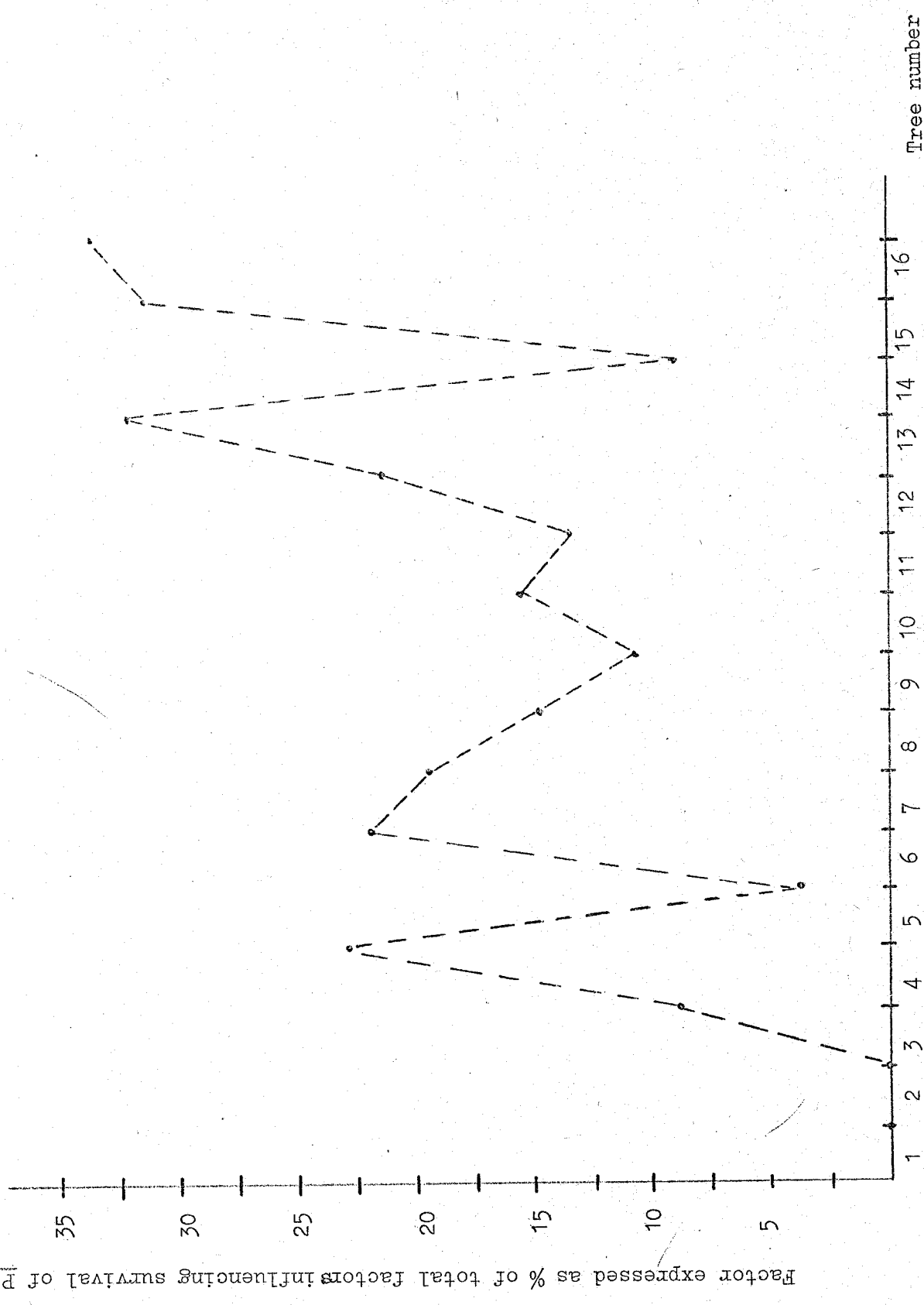
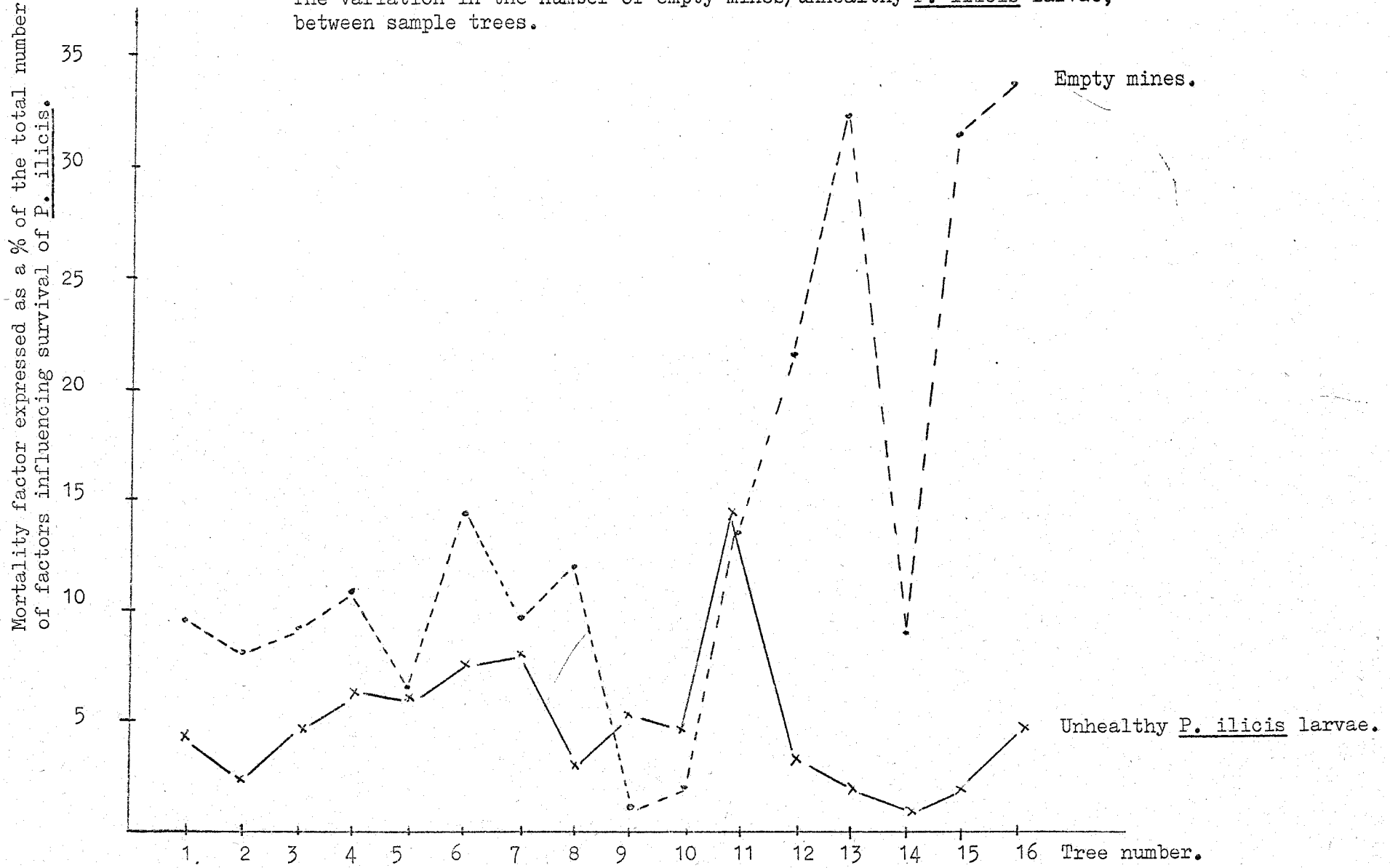


FIG. 15
The variation in the number of empty mines/unhealthy P. ilicis larvae, between sample trees.



Thus these trees would appear to have suffered more merely because they were exposed for a longer period before any measurements were taken. From Fig.13 , however this seems unlikely.

The calculated X^2 for variation in bird-feeding between trees was 22.85 (Appendix4). The tabulated value at 5% significance was 25, thus no significant difference between trees was observed. The difference for C. gemma between trees was significant. The calculated X^2 of 32.37 was significant at 1% level. No significant difference between trees with regard to unknown mortality was observed. A calculated value for X^2 of 17.24 was obtained. Significant differences between trees for larvae to pupae survival was obtained at 5% level i.e. calculated X^2 of 25.43 compared with tabulated values of 25, although this was insignificant at 1% level. These calculations were based on Table22 , where results were expressed as values per 100 leaves. This method of expression was good for total egg number and mine number data, however since the number of bird-pecks, and attack by larval parasites, depended on the number of eggs surviving to produce mines i.e. total mine number per tree, it was better to express these values as % results for total mine number as in Table21. Where % values were compared, significant differences were obtained for all parameters between trees, and this was assumed to be the most accurate representation of the data.

Larvae surviving to the pupal stage, apart from further attack by feeding birds are liable to attack by pupal parasites. The species of parasites observed during this investigation were Chrysocharis syma and Sphegigaster flavicornis. C. syma was found from the mature larval stage to the adult stage only. S. flavicornis was recorded from the mature - prepupal stage. A detailed discussion will be given later.

A summary for parasite species and numbers of individual parasites recorded for each tree is given in Table23 , together with values for non-parasitised pupae. Each figure is expressed as a % of the total recordings. The variation in the extent of attack by each species for the trees examined is given in Fig.16 . C. syma is the most important pupal parasite and was recorded from all trees. Highest figures for parasitism by this species was for Trees 4 and 6, where values of 55.56 and 56.86 were obtained. Lowest incidence was observed for Trees 1, 7 and 8, % parasitism being 4.03, 11.76 and 5.8% respectively. The % parasitism by Sphegigaster flavicornis fluctuated between trees, having least effect on pupae extracted from Trees 1,14,15 and 16. Highest levels of attack were recorded for Trees 4,5,6,9,10 and 11 where values of 18.52, 21.05, 25.49, 25.80, 29.41 and 31.25 were obtained. % pupal parasitism recorded for Trees 1,2,3,5,6,7,14,15 and 16 fell between 0 - 50%. Values between 50% and 82%, were observed for remaining trees. Differences between trees was tested using X^2 tests and significant differences were observed at 1% level.

A summary of % pupae resisting parasitism is outlined in Table 23. Healthiest pupae were recorded from Trees 1,2,7 and 8, least pupal survival being observed from Trees 4,6,9,10 and 11.

Table 22.

Summary Sheet for Trees sampled 1 - 16

Tree No.	Eggs per 100 Leaves	Mines per 100 Leaves	Bird-Pecks per 100 Leaves	C. gemma per 100 Leaves	Emptymineper 100 Leaves	Larvae Pupae per 100 Leaves
1.	13.42	10.94	5.63	0	0.93	3.99
2.	10.77	8.96	6.29	0	0.650	1.81
3.	25.70	18.60	10.67	1.65	1.72	3.79
4.	18.58	14.69	4.69	3.15	1.54	3.62
5.	33.24	23.92	12.51	0.815	1.69	5.53
6.	32.06	25.28	5.02	5.58	3.62	9.48
7.	23.70	19.08	7.08	3.69	1.85	5.23
8.	14.08	9.53	4.27	1.42	1.14	2.42
9.	18.71	13.29	6.57	1.43	0.14	4.43
10.	31.37	22.37	11.11	3.55	5.26	4.52
11.	13.06	9.94	2.37	1.34	2.37	2.37
12.	30.93	21.49	7.08	4.60	5.31	3.54
13.	15.83	12.61	3.08	4.06	1.82	3.36
14.	28.21	22.01	5.48	1.98	1. 0	12.25
15.	40.21	29.25	4.64	9.15	4.90	9.79
16.	20.84	20.65	1.74	6.97	4.22	6.72

Table 23.

Summary for parasite species and numbers attacking P. ilicis pupae.

Parasites		Expressed as % of total
Chrysocharis	5	4.03
Sphegigaster flavicornis	4	3.23
Unidentified parasites	3	2.42
Non-parasitized pupae	112	90.32

TREE 1.

Parasites		Expressed as % of total
Chrysocharis	7	28.00
Sphegigaster flavicornis	2	8.00
Unidentified parasites	0	0
Non-parasitized pupae	16	64.00

TREE 2.

Parasites		Expressed as % of total
C. syma	45	27.61
S. flavicornis	14	
Unidentified Parasites	3	1.84
Non-parasitized pupae	101	61.96

TREE 3.

Parasites		Expressed as % of total
C. syma	30	55.50
S. flavicornis	10	18.52
Unidentified Parasites	3	7.41
Non-parasitized pupae	10	18.52

TREE 4.

Parasites		Expressed as % of total
C. suma	26	27.37
S. flavicornis	20	21.05
Unidentified parasites	0	0
Non-parasitized pupae	49	51.58

TREE 5.

Parasites		Expressed as % of total
C. syma	58	56,86
S. flavicornis	26	25.49
Unidentified parasites		
Non-parasitized pupae	18	17.65

TREE 6.

Parasites		Expressed as % of leaf total
C. syma	2	11.76
S. Flavicornis	2	11.76
Unidentified parasites	-	
Non-parasitized pupae	13	76.47

TREE 7.

Parasites		Expressed as % of leaf total
C. syma	1 (2)	5.8 (11.6)
S. Flavicornis	1 (2)	5.8 (11.6)
Unidentified parasites	0	0
Non-parasitized pupae	15	88.24

TREE 8.

Parasites		Expressed as % of total
C. syma	24	88.70
S. Flavicornins	16	25.80
Unidentified parasites	0	0
Non-parasitized pupae	22	35.48

TREE 9.

Parasites		Expressed as % of total
C. syma	14	40.20
S. Flavicornis	30	29.41
Unidentified parasites	2	1.96
Non-parasitized pupae	29	28.43

TREE 10.

Parasites		Expressed as % of total
C. syma	6	37.50
S. Flavicornis	5	31.25
Unidentified parasites	-	
Non-parasitized pupae	5	31.25

TREE 11.

Parasites		Expressed as % of total
C. syma	14	46.67
S. Flavicornis	5	16.67
Unidentified parasites	-	-
Non-parasitized pupae	11	36.67

TREE 12.

Parasites		Expressed as % of total
C. syma	9	37.50
S. flavicornis	4	16.67
Unidentified parasites		-
Non-parasitized pupae	11	45.80

TREE 13.

Parasites		Expressed as % of total
C. syma	57	40.71
S. flavicornis	25	1.79
Unidentified parasites	-0	
Non-parasitized pupae	58	41.43

TREE 14.

Parasites		Expressed as % of total
C. syma	33	43.42
S. flavicornis	2	2.63
Unidentified parasites	-	
Non-parasitized pupae	41	53.95

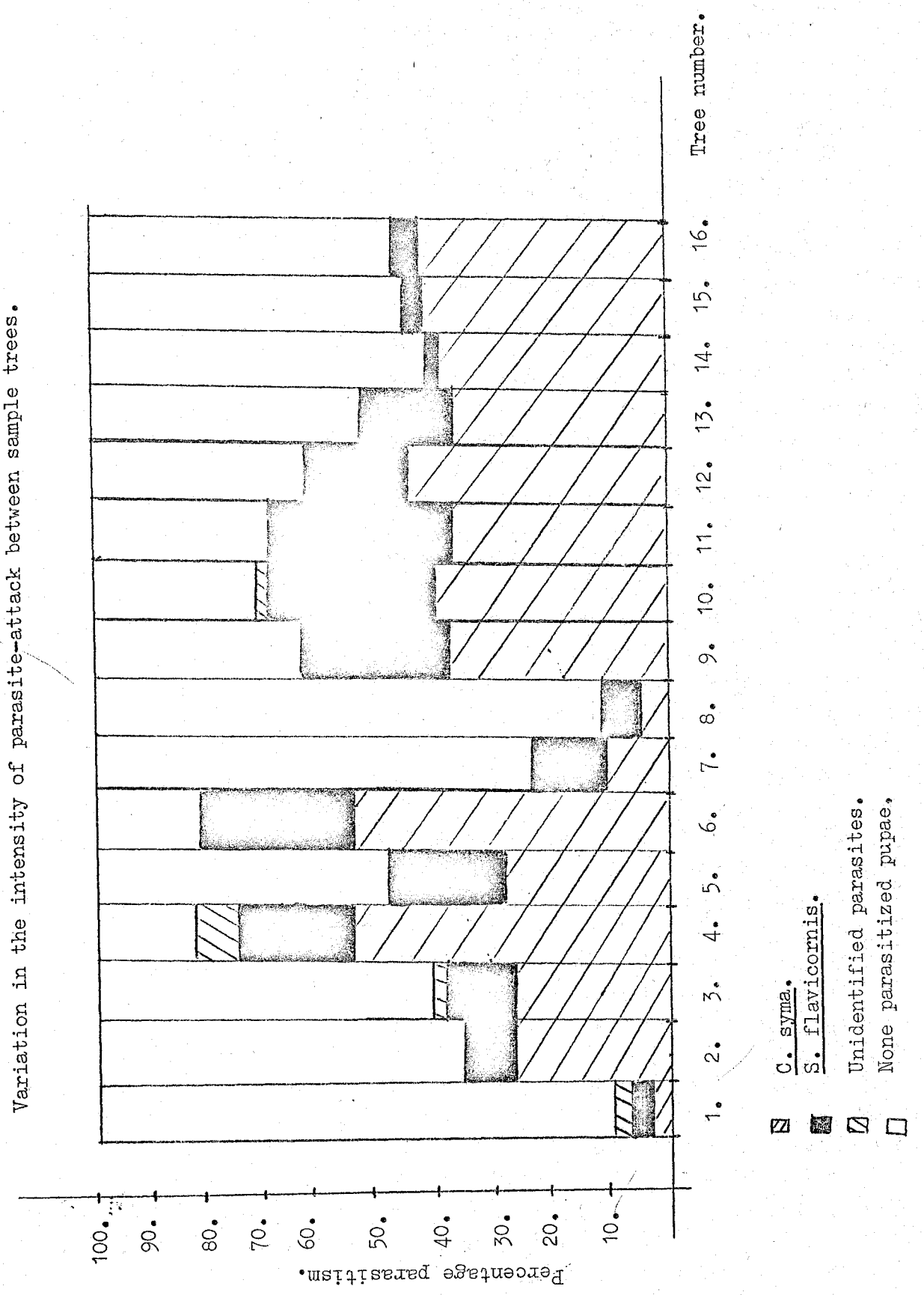
TREE 15.

Parasites		Expressed as % of total
C. syma	12	44.44
S. flavicornis	1	3.70
Unidentified parasites	-	
Non-parasitized pupae	14	51.85

TREE 16.

FIG. 16

Variation in the intensity of parasite-attack between sample trees.



Thus sumarizing for all information obtained, and taking total egg number as estimates of population density per tree, for Tree 1 for 100 eggs originally laid :-

1. 18.47 succumbed to egg mortality.
2. 41.97 were eaten by birds.
3. 9.83 died due to unknown causes.
4. 2.88 were killed by pupal parasites.
5. 26.86 were assumed to emerge successfully.

Similar data was obtained for other study trees and summarized in Table 24. Hence after all mortality factors have been accounted for, those pupae unaffected could be assumed to emerge successfully. This assumption may over estimate emergence since it is possible that further mortality will be encured especially by bird-attack. Values obtained for trees studied later in the sampling program were more accurate since P. ilicis had begun to emerge and actual values were recorded and used in comparisons. For most trees sampling was completed before adult emergence, hence ideally, later samples for all trees should have been taken, but due to a time restriction this was not feasible. The final sample was taken for Tree 14 on 17.7.78, and at this point 46 from a possible 58 pupae emerged, and 3 of the remaining pupae were dead. According to Miall and Taylor (1908), adult Phytomyza were abundant throughout June thus it is difficult to postulate whether remaining pupae are later in development because of the less favourable climate experienced in Northern areas when compared with sites studied by the forementioned workers, or whether these pupae are unhealthy hence will fail to complete development regardless. Adults were recorded for Trees 12,14,15, and 16, i.e. from 12.7.78 onwards. The actual recordings for emergence are given in Table 24. Thus by comparing the values for assumed P. ilicis emergence, after all mortality factors have operated, excluding Tree 1, the values range between 3.42 and 18.47%, a significant difference at both 5% and 1% levels existing. Most flies were assumed to emerge from Trees 1,7, and 14, thus for Tree 1 even though infestation was low, the number of successful emerging adults was the highest.

Table 24

SUMMARY OF FACTOR INFLUENCING THE SURVIVAL OF 100 EGGS OF P.ILICIS SAMPLED PER TREE

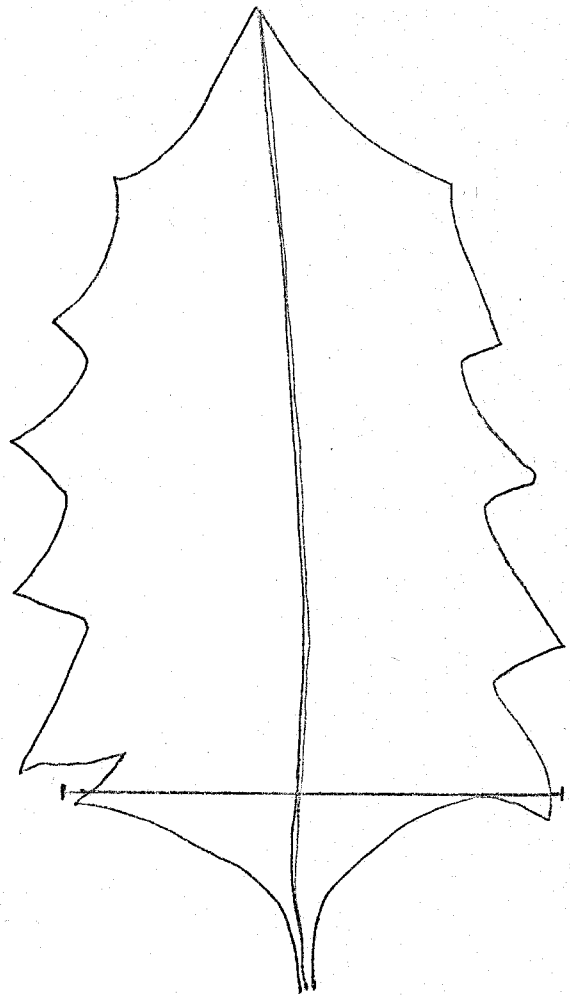
TREE No.	EGG MORTALITY	EATEN BY BIRDS	DIED DUE TO UNKNOWN CAUSES	KILLED BY LARVAL PARASITES	KILLED BY PUPAL PARASITES	THOSE ASSUMED TO EMERGE	ACTUAL RECORDINGS TO DATE OF SAMPLING FOR EMERGENCE
1	18.47	41.97	9.83	0	2.88	26.86	-
2	16.78	58.39	8.05	0	6.04	10.74	-
3	27.24	41.54	9.68	6.43	5.61	9.14	-
4	20.93	25.27	13.00	16.97	15.88	3.61	-
5	28.02	37.65	9.11	2.45	8.06	8.58	-
6	21.16	15.65	16.23	17.39	24.35	5.22	-
7	20.83	29.87	14.29	15.58	5.19	16.88	-
8	32.32	30.30	10.10	10.10	2.02	15.15	-
9	29.01	35.11	4.58	7.63	15.27	8.40	-
10	28.15	35.42	19.95	11.33	8.62	3.42	-
11	23.86	18.18	29.55	1.02	12.50	5.62	-
12	30.53	22.90	1.91	14.89	7.25	4.20	4.00 12/7/78
13	20.53	19.47	13.27	25.56	11.50	9.73	
14	21.97	19.43	7.01	7.01	26.11	18.47	14.33 17/7/78
15	27.24	11.54	13.78	22.76	11.22	13.14	4.49 13/7/78
16	8.79	7.69	23.08	30.77	14.39	15.38	2.02 13/7/78

4.4. Leaf section results.

The results of various parameters - leaf length, width, and leaf thickness, measured at three sites a,b, and c on the leaf corresponding to the midrib and two points either side of the midrib, within the leaf blade is given in Table 25 . These points corresponded to egg-laying sites and areas where leaf piercing was common. Measurements for cuticle thickness at these sites for both the upper and lower epidermis is also given in Table 25 . All measurements are in mm, and the leaves were sampled from the same trees. Fig.18 indicates leaf length plotted against leaf width. . These were significantly correlated i.e. r = 0.9181. The straight line equation obtained was $y = 0.67\bar{x} - 2.5$. For leaf thickness measurements taken in the leaf blade plotted against leaf length (Fig.19), a significant correlation was obtained. Values for the straight line equation were $y = 128.14x - 6.64$, when r = 0.51. For leaf thickness measured across the midrib, the straight line equation obtained was $y = 58.36x - 7.48$, with r = 0.78. Thus it would appear that leaf length and midrib thickness are more strongly associated. When leaf thickness was plotted against leaf width, r was 0.52 for measurements of leaf thickness taken within the leaf blade, and r was 0.67 when measured at the midrib. Values for the straight line equation obtained were $y = 120x - 14.48$ and $y = 36.77x - 5.81$ respectively (Fig 20). Leaf length was plotted against cuticle thickness at the three sites for both the upper and lower epidermis (Fig.21,22),(Table 25 .) The mean values for cuticle thickness at the midrib for the upper and lower epidermis using the range of leaves provided in Table 25were compared. The mean values were equivalent, and any differences in individual recordings were insignificant. Thus an r value for leaf length plotted against cuticle thickness for the upper epidermis only was obtained. Leaf length and cuticle thickness at both the midrib and within the leaf were not significantly correlated. The r value for cuticle thickness at the midrib against leaf length was 0.301. An r value of 0.08 was obtained for cuticle thickness within the leaf blade plotted against leaf length (Fig. 23). Where leaf thickness was plotted against cuticle thickness at the midrib, an insignificant result was obtained i.e. r = 0.108 (Fig. 24). Thus for the size range of leaves sampled, leaf size and leaf thickness were found to be significantly correlated, whilst cuticle thickness is independant of leaf size despite the trend of increasing cuticle thickness with leaf size as indicated in Figs.21,22,23,24.

Leaves of the current seasons growth were collected on 22.6.78, when leaf expansion was on the increase. Leaf measurements were obtained as described earlier. Results for leaf length, width, leaf thickness measured at the three forementioned sites, and cuticle thickness for the upper epidermis only are given in Table 26. The values for sites a and c were bulked and the mean values obtained. For group 1, it was difficult to measure the cuticle thickness since the leaves were young and fragile and all differentiation was in the early stages. Five leaves were sampled per group and mean values obtained. From Table 26, as leaf size increases the leaf expands and the cuticle thickness increases both in the leaf blade and across the midrib. Fig.25 indicates how the cuticle developes with increase in age and size.

Fig.17 Leaf-Sections



Section taken across leaf.

Areas where measurements taken.

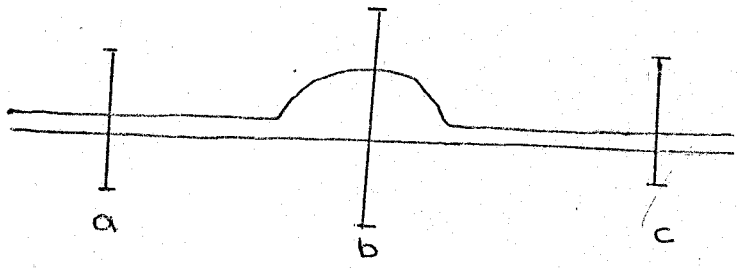


Fig 18. Leaf width plotted against leaf length.

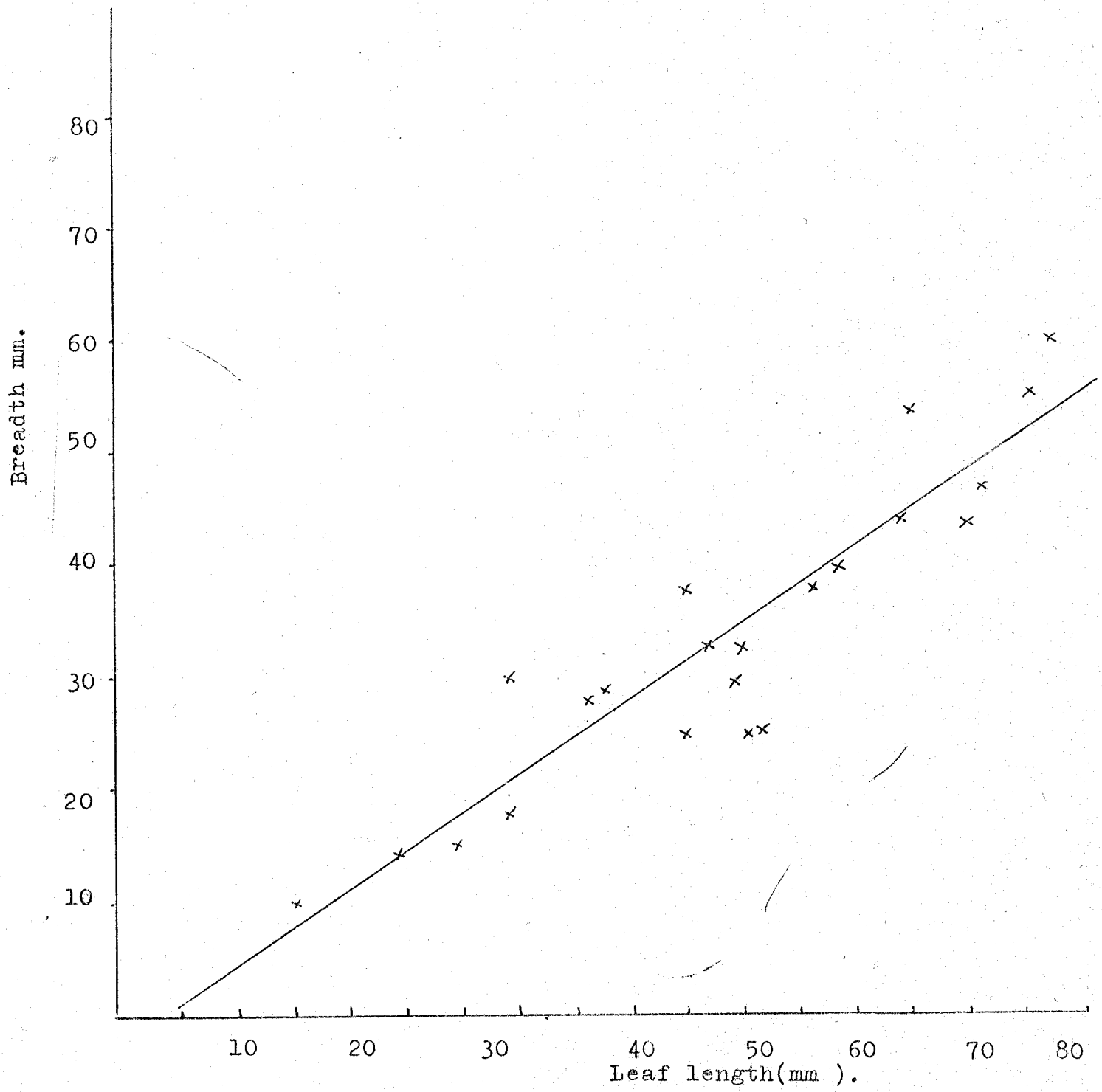


Fig19. Length of leaves plotted against mean leaf thickness across leaf either side of midrib, (1)/against leaf thickness at the midrib, (2).

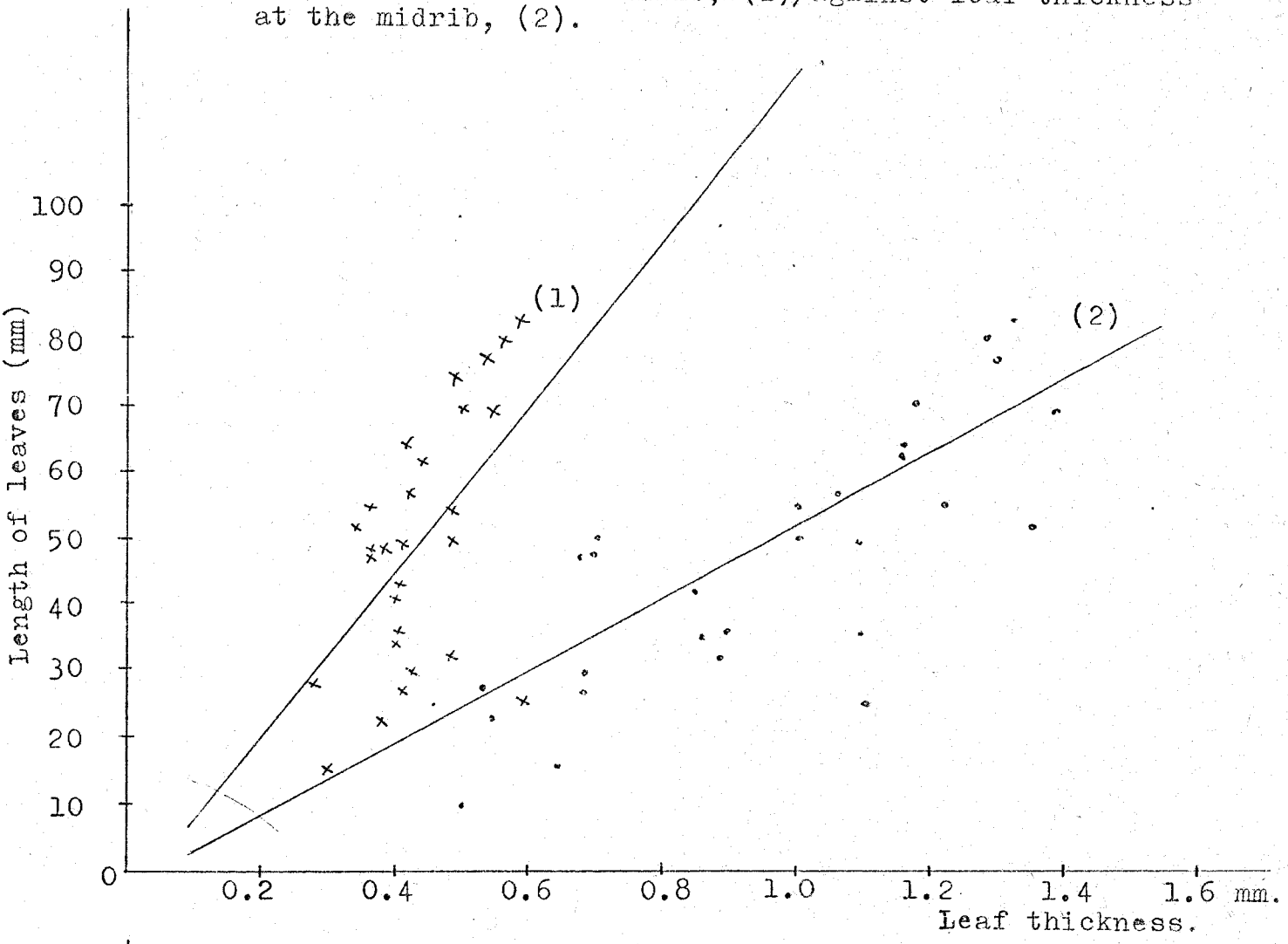


Fig 20.
Leaf width plotted against mean leaf thickness for (1 /2)

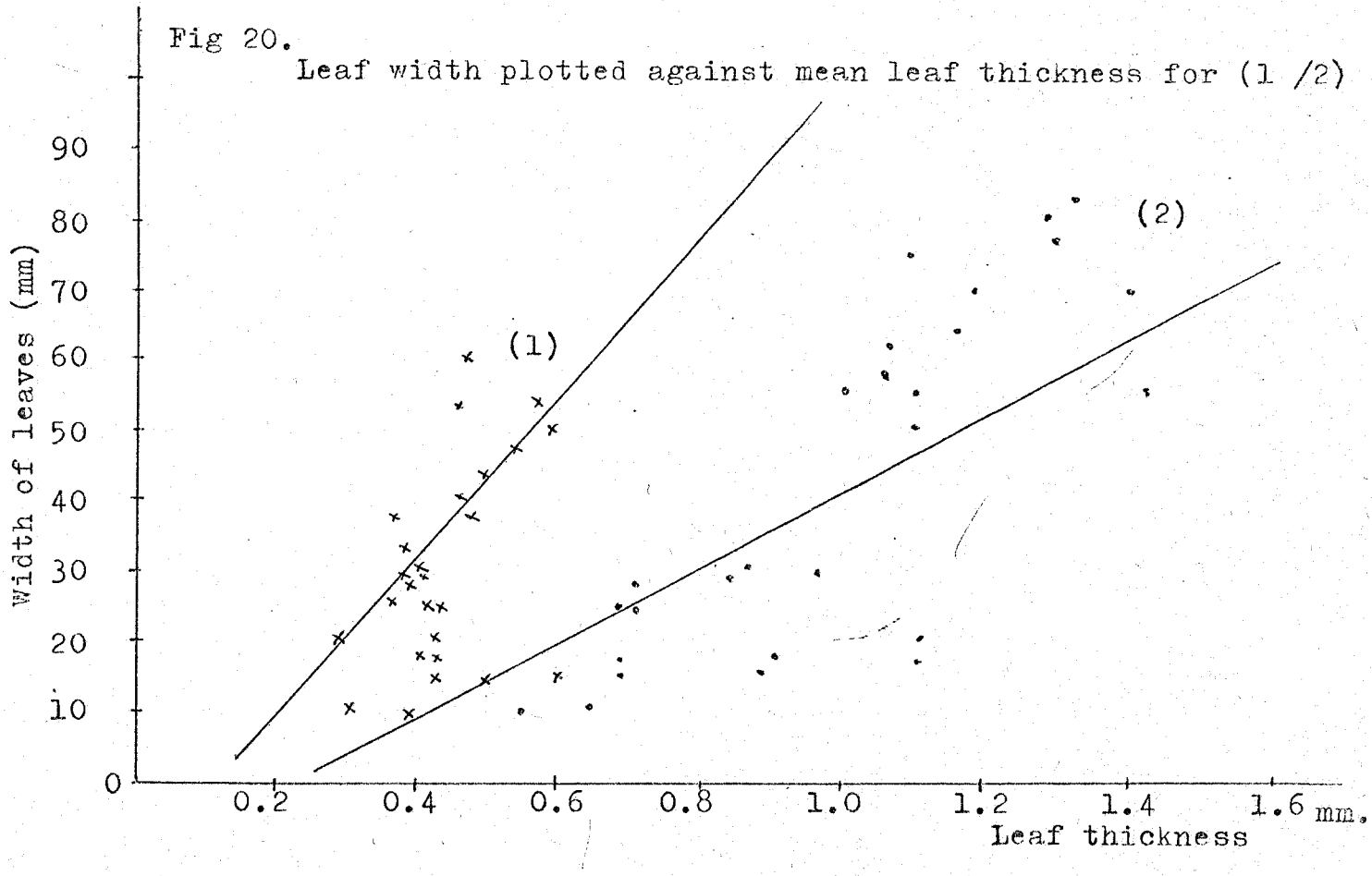


Fig 21a. Leaf length plotted against cuticle thickness (upper epidermis.)

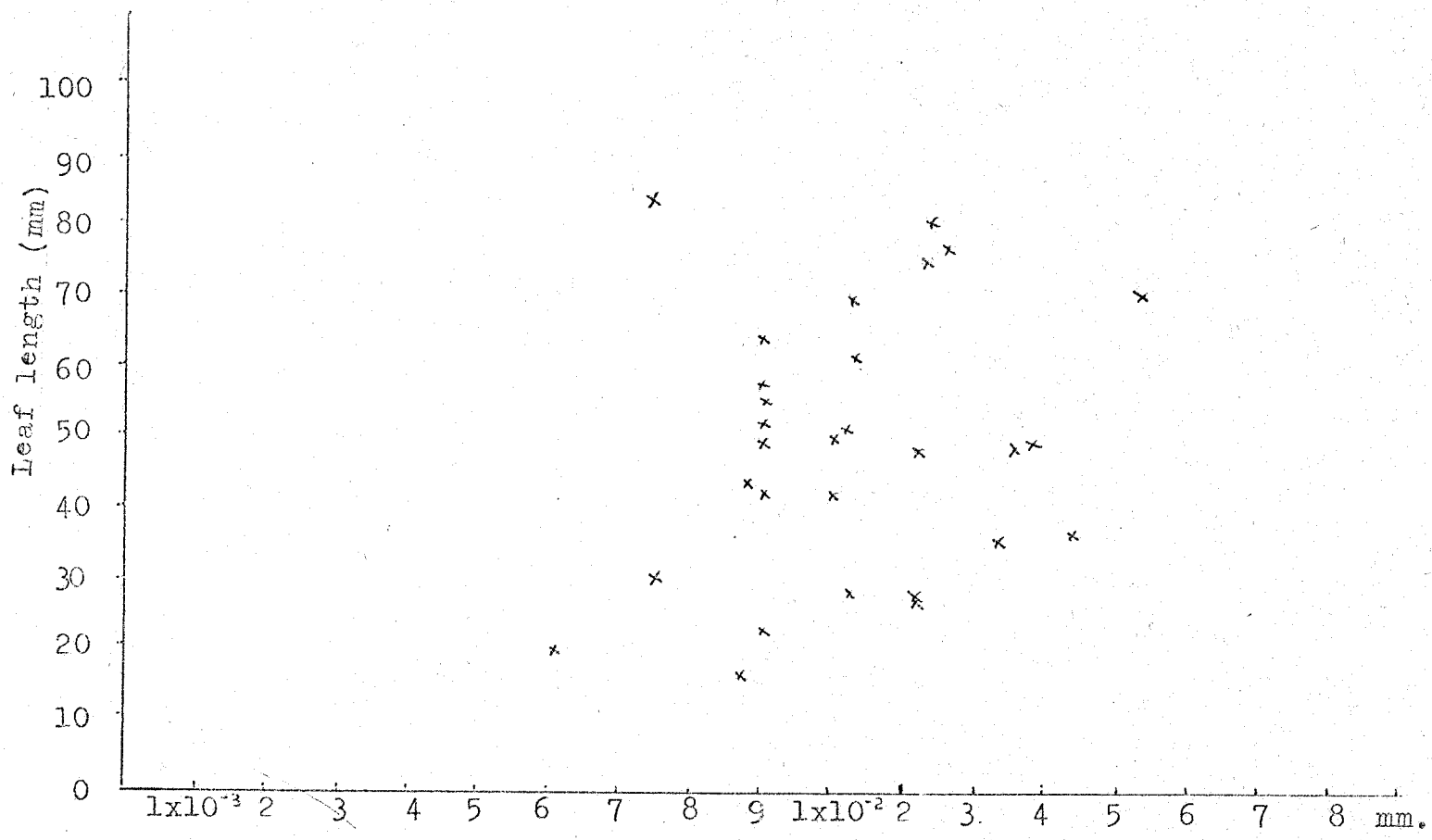


Fig 21b. Leaf length plotted against cuticle thickness (lower epidermis)

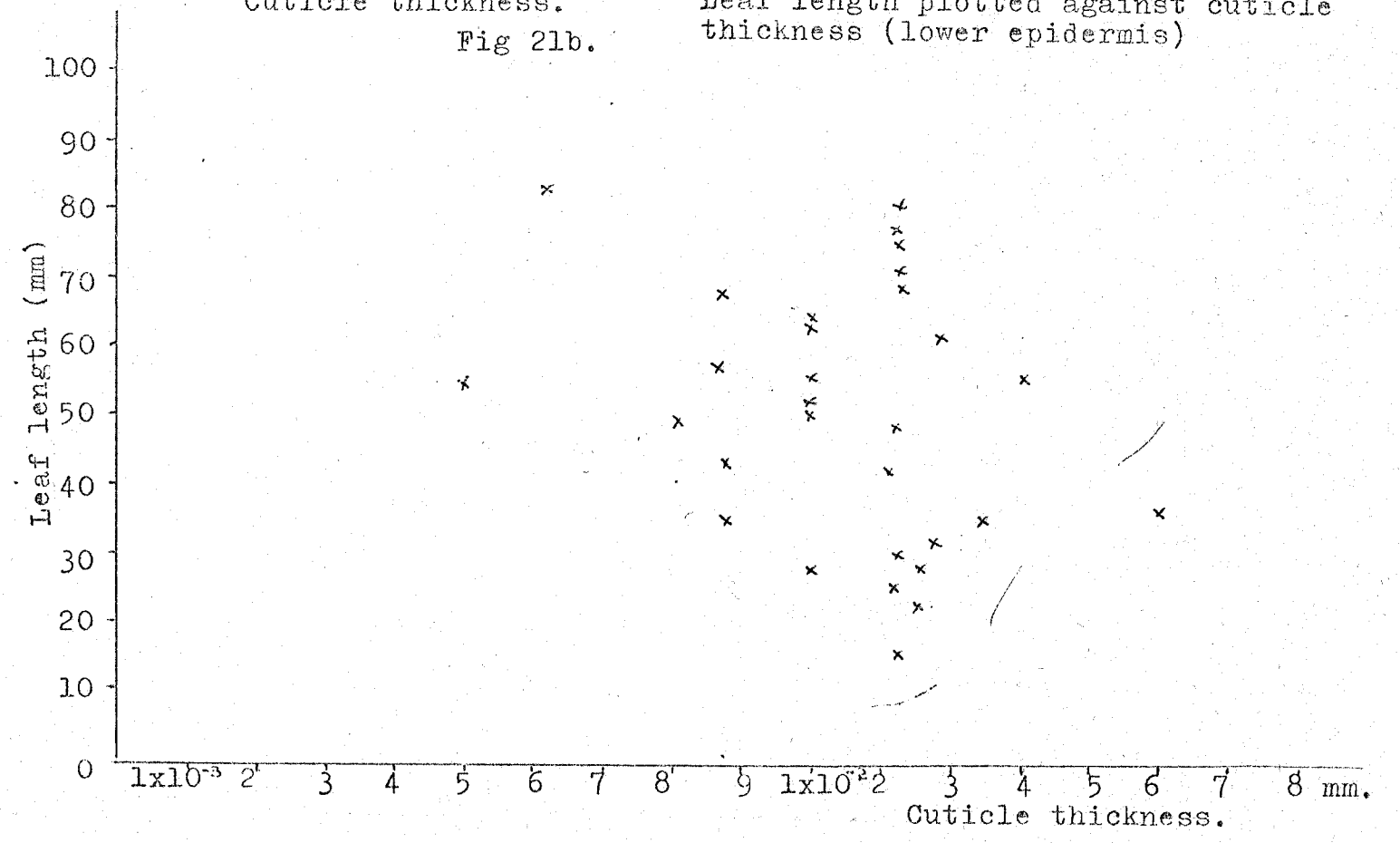


Fig.22a. Leaf length plotted against cuticle thickness at the midrib for lower and upper epidermis.

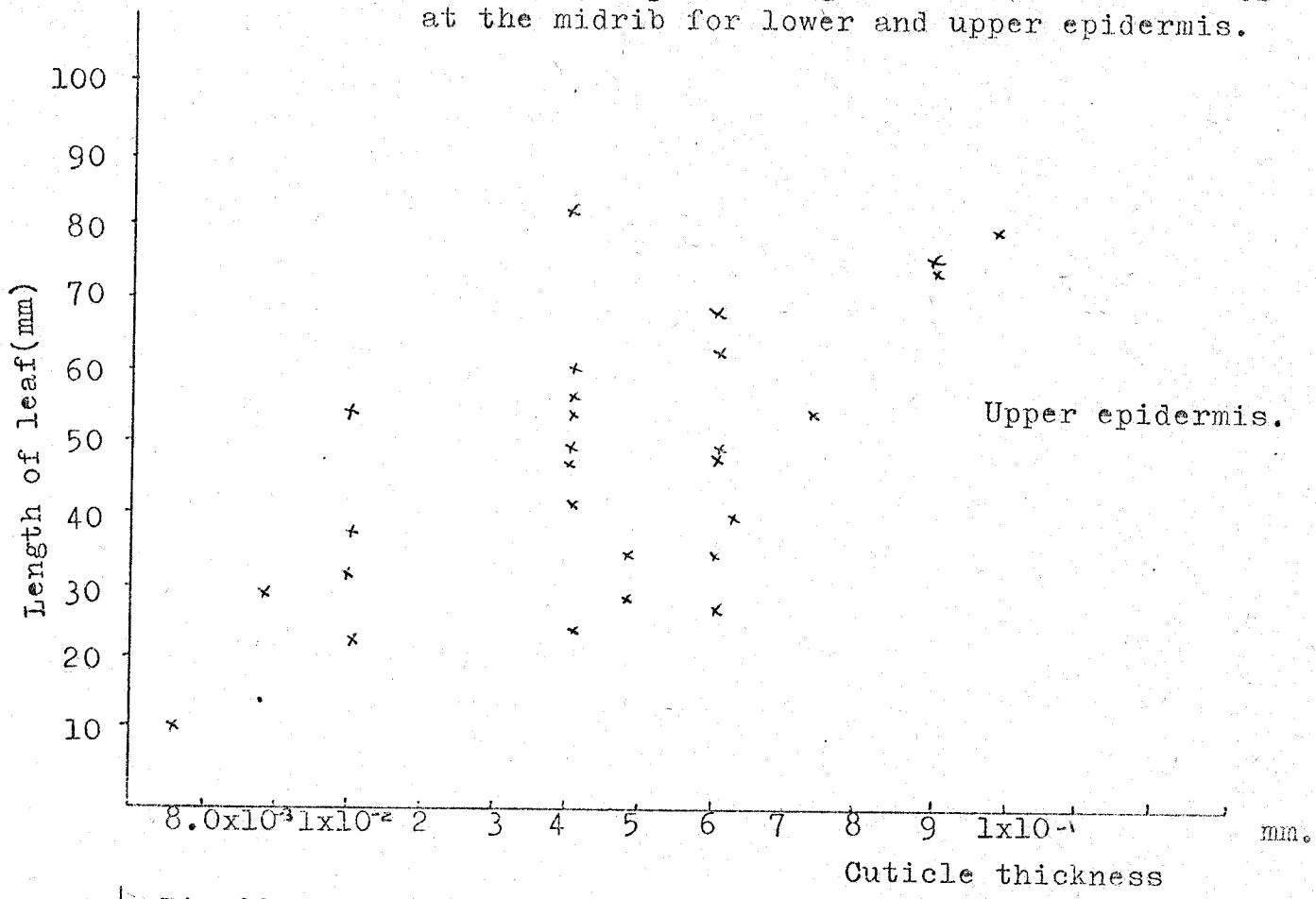


Fig 22b.

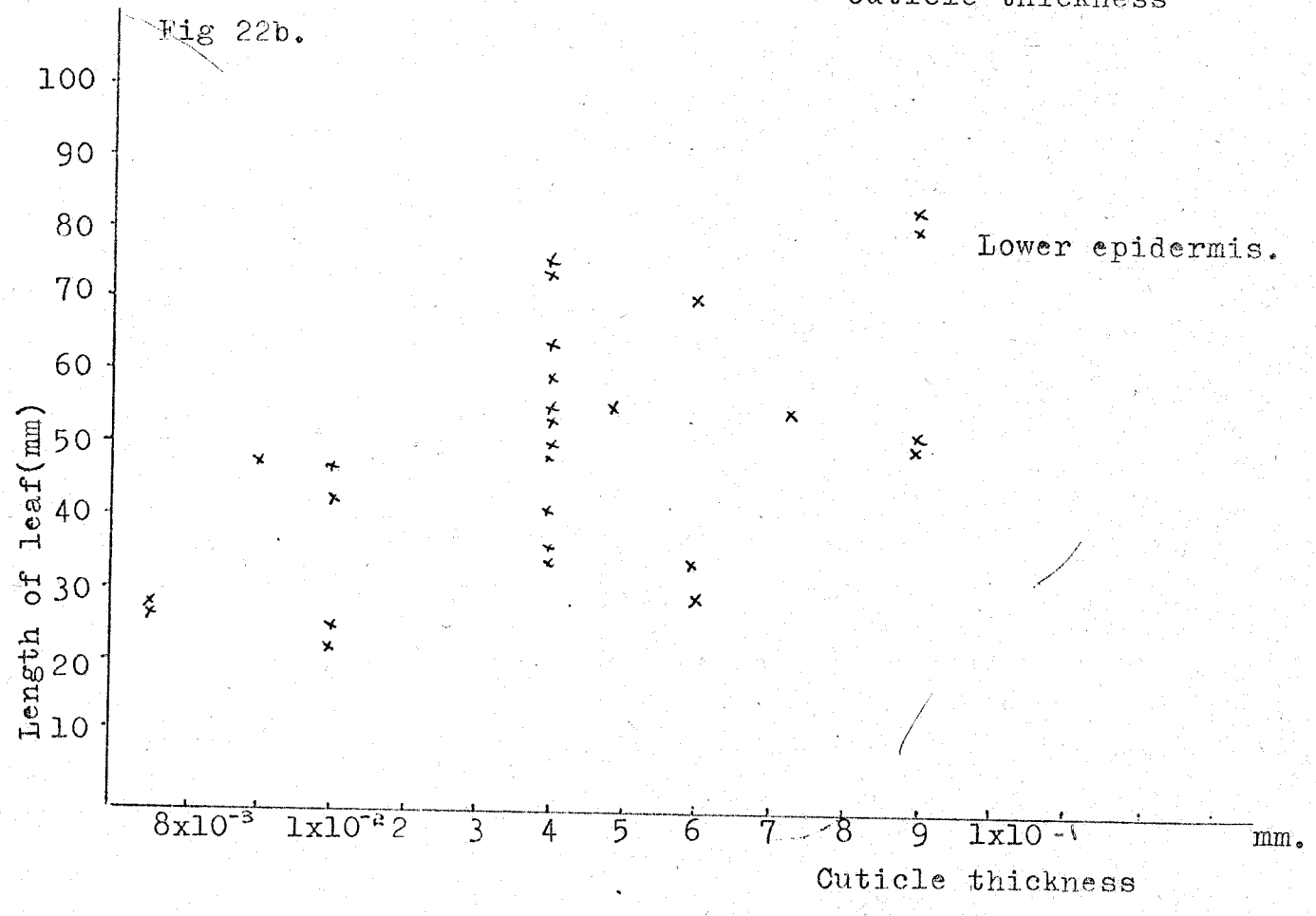


Fig23 Cuticle thickness plotted against leaf length(blade)

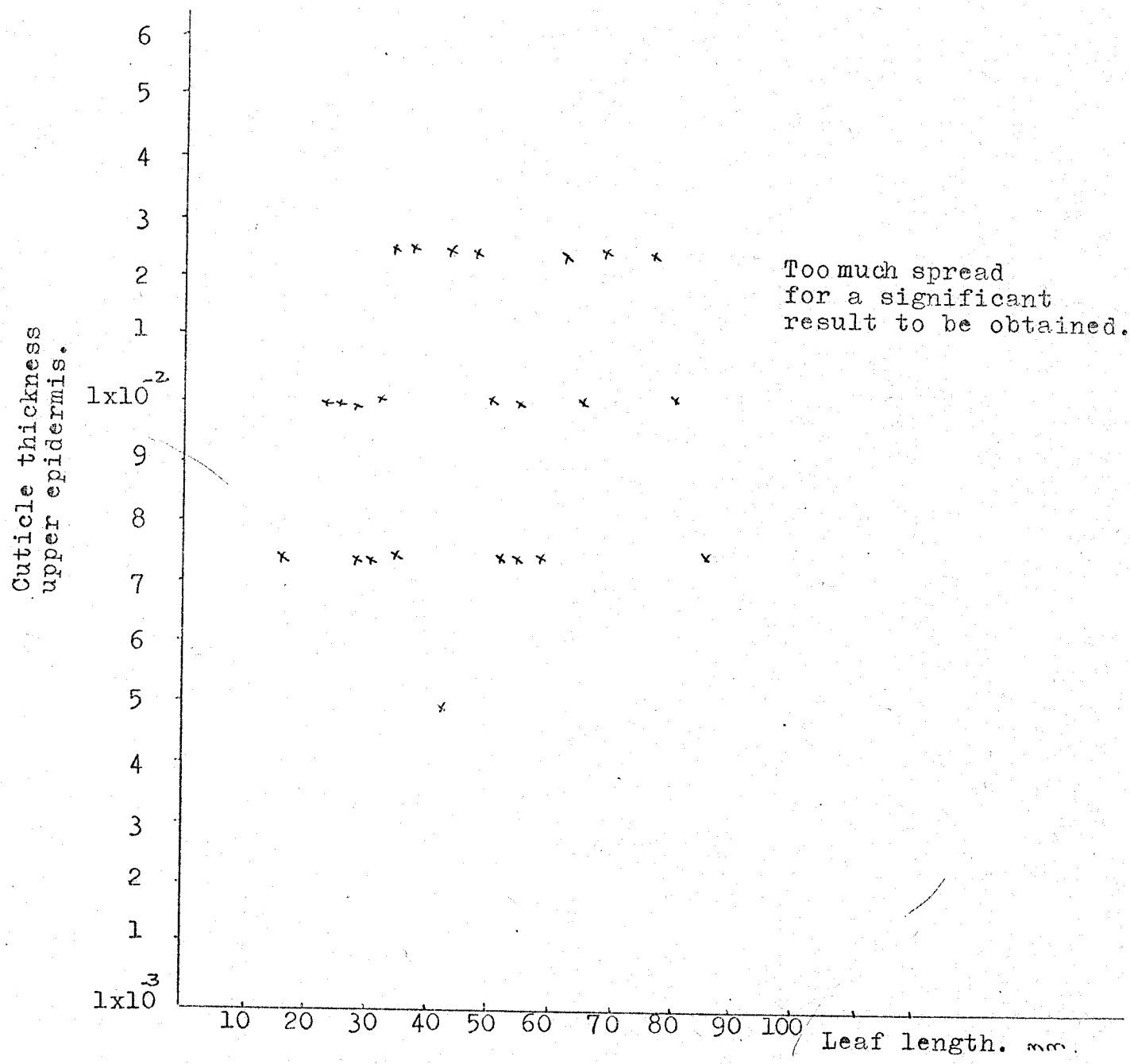


Fig. 24. Leaf thickness plotted against cuticle thickness. (across midrib).

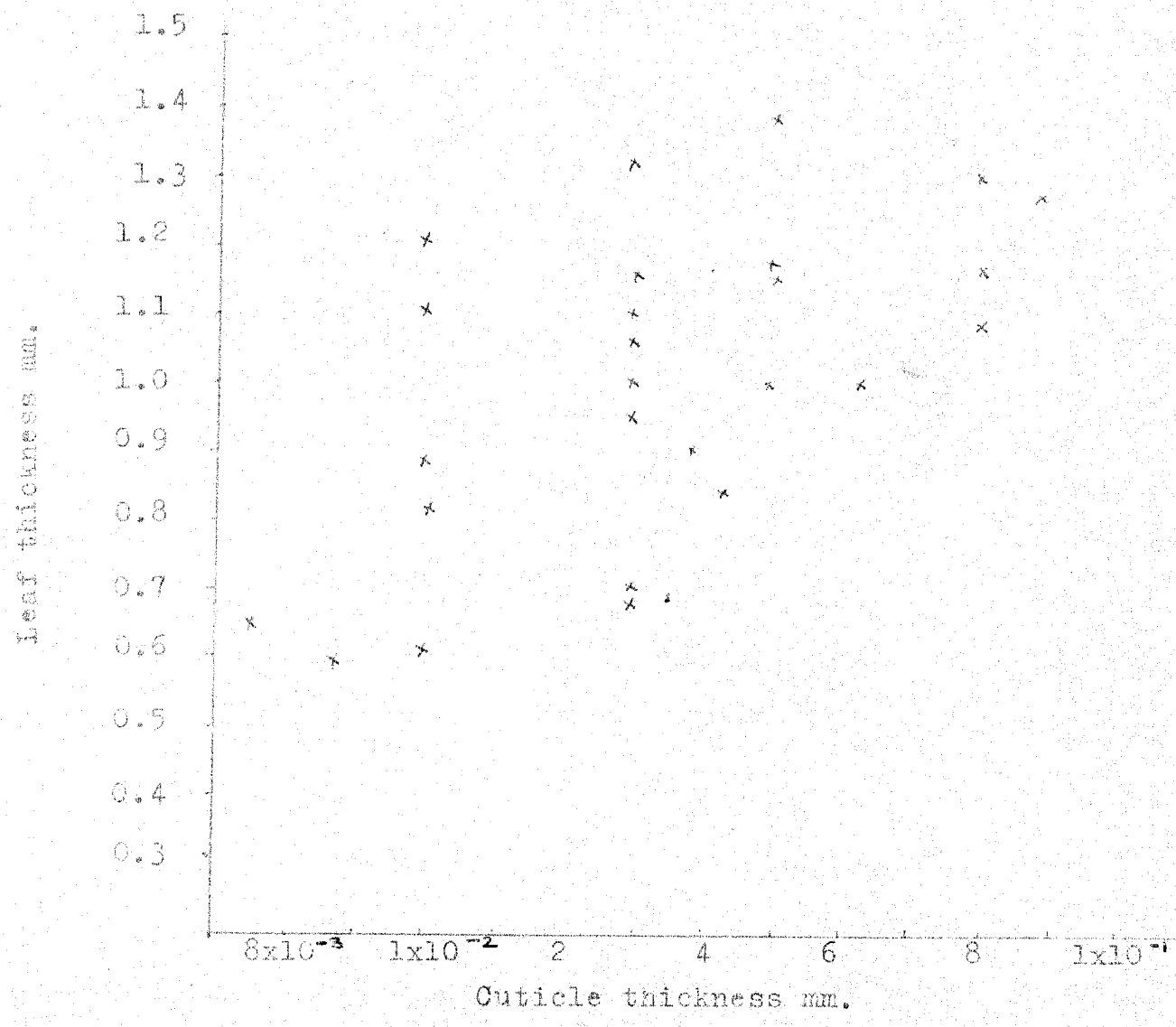


Table 25.

Leaf Blade, Midrib and Cuticle Thickness (Measurement in mm)

Leaf Thickness					Cuticle Thickness					
					Upper Epidermis			Lower Epidermis		
Length	Width	a	b	c	a	b	c	a	b	c
35	18	0.4	0.9	0.4	1.25×10^{-2}	1.38×10^{-2}	1×10^{-2}	1.25×10^{-2}	15×10^{-2}	1.25×10^{-2}
50	25	0.4	0.7	0.42	1.0×10^{-2}	1.25×10^{-2}	1×10^{-2}	1.0×10^{-2}	1.25×10^{-2}	1×10^{-2}
42	28	0.4	0.84	0.4	1.25×10^{-2}	1.53×10^{-2}	1.5×10^{-3}	1×10^{-2}	1.25×10^{-2}	1.25×10^{-2}
43	29	0.4	0.96	0.4	5×10^{-3}	1.25×10^{-2}	1.25^{-2}	7.5×10^{-3}	1.0×10^{-2}	1.0×10^{-2}
35	30	0.4	0.86	0.4	7.5×10^{-3}	10×10^{-2}	7.5×10^{-3}	1.0×10^{-2}	1.25×10^{-2}	7.5×10^{-3}
55	30	0.46	1.22	0.50	1×10^{-2}	1×10^{-2}	1×10^{-2}	1.25×10^{-2}	1.38^{-2}	1.13×10^{-2}
50	38	0.4	1.00	0.54	1×10^{-2}	$1.5x^{-2}$	1.13^{-2}	1.0×10^{-2}	1.8^{-2}	1.0^{-2}
55	30	0.48	1.00	0.48	7.5^{-3}	1.63^{-2}	1.13^{-2}	1.25^{-2}	1.62^{-2}	1.5^{-2}
52	33	0.4	1.14	0.36	7.5×10^{-3}	8.8^{-3}	1.0^{-2}	1.25^{-2}	1.8^{-2}	1.0^{-2}
57	25	0.4	1.06	0.44	7.5×10^{-3}	1.25^{-2}	1.0^{-2}	1.25^{-2}	1.25^{-2}	7.5×10^{-3}
55	38	0.36	1.00	0.36	10×10^{-2}	1.25^{-2}	1.0^{-2}	5×10^{-3}	1.25^{-2}	5×10^{-3}
77	47	0.56	1.30	0.50	1.25^{-2}	1.8^{-2}	1.25^{-2}	1.25^{-2}	1.25^{-2}	1.0^{-2}
64	40	0.48	1.16	0.44	1.0^{-2}	1.5^{-2}	1.0^{-2}	1.25^{-2}	1.25^{-2}	7.5^{-3}
69	44	0.52	1.38	0.64	1.25^{-2}	1.5^{-2}	1.0^{-2}	1.0^{-2}	1.25^{-2}	1.25^{-2}
62	38	0.44	1.16	0.50	1.25^{-2}	1.25^{-2}	1.0^{-2}	1.13^{-2}	1.25^{-2}	1.25^{-2}
83	60	0.5	1.32	0.46	7.5^{-3}	1.25^{-2}	7.5^{-3}	7.5^{-3}	1.8^{-2}	5.0^{-3}
70	54	0.44	1.18	0.46	1.8^{-2}	1.5^{-2}	1.25^{-2}	1.25^{-2}	1.5^{-2}	1.0^{-2}

Continued

Leaf Thickness					Cuticle Thickness					
					Upper Epidermis			Lower Epidermis		
Length	Width	a	b	c	a	b	c	a	b	c
80	55	0.56	1.28	0.56	1.0^{-2}	1.88^{-2}	1.25^{-2}	1.25^{-2}	1.8^{-2}	1.0^{-2}
75	44	0.48	1.09	0.5	1.25^{-2}	1.8^{-2}	10^{-2}	1.25^{-2}	1.25^{-2}	1.0^{-2}
30	15	0.42	0.68	0.42	0.0075	8.75×10^{-3}	8.75×10^{-3}	1.125×10^{-2}	1.5×10^{-2}	0.01
25	15	0.48	1.10	0.70	0.01	0.01	0.0125	0.01	0.01	0,0125
27	17	0.44	0.68	0.38	0.01	0.01	0.0138	0.0125	0.0075	0.0125
16	10	0.34	0.64	0.26	0.0075	0.0075	0.01	0.0125	0.005	0.0015
23	10	0.38	0.54	0.38	0.01	0.015	0.01	0.01	0.01	0.015
28	20	0.28	0.530	0.28	0.0025	0.0113	0.015	0.0125	0.0075	0.0125
36	20	0.4	1.10	0.44	0.0125	0.015	0.0150	0.0175	0.0125	0.0125
48	28	0.36	0.70	0.40	0.0125	0.0125	0.01	0.0125	0.009	0.010
48	25	0.36	0.68	0.36	0.0125	0.015	0.0125	0.0125	0.01	0.01
32	15	0.42	0.88	0.54	0.01	0.01	0.015	0.0138	0.0125	0.01

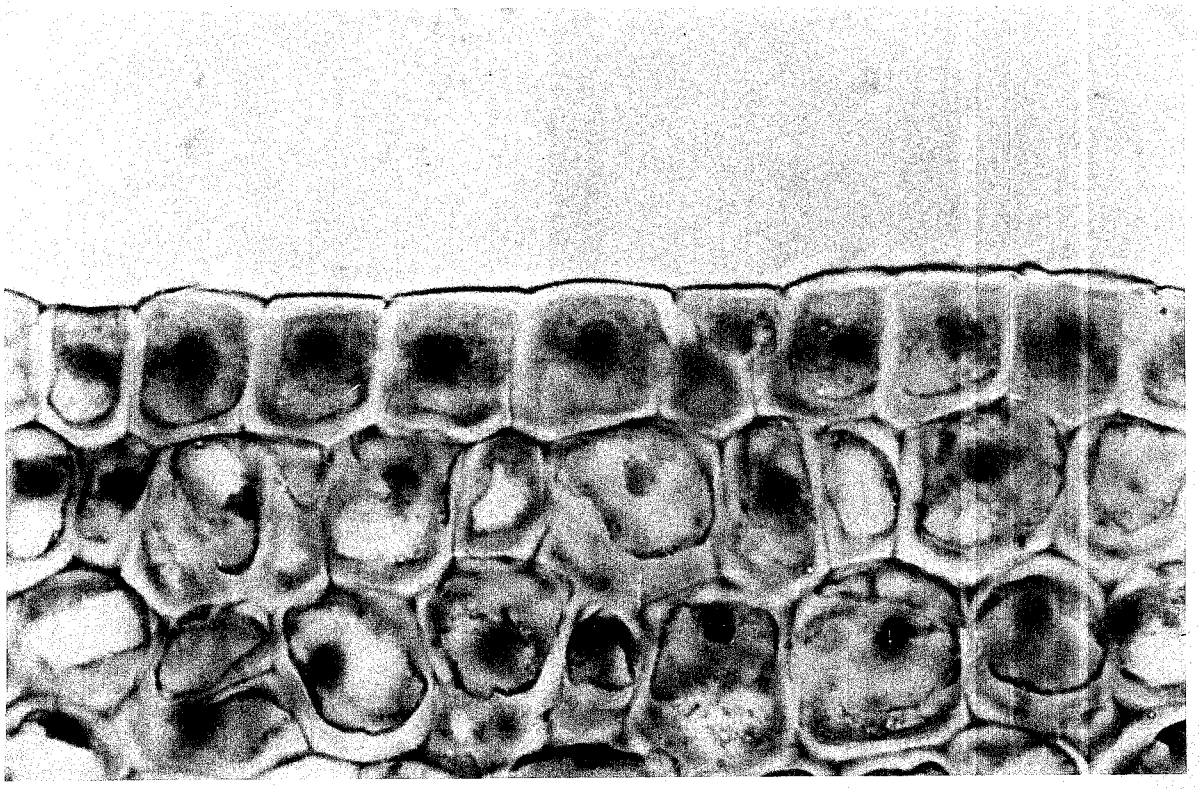
Table 25.

Table 26.

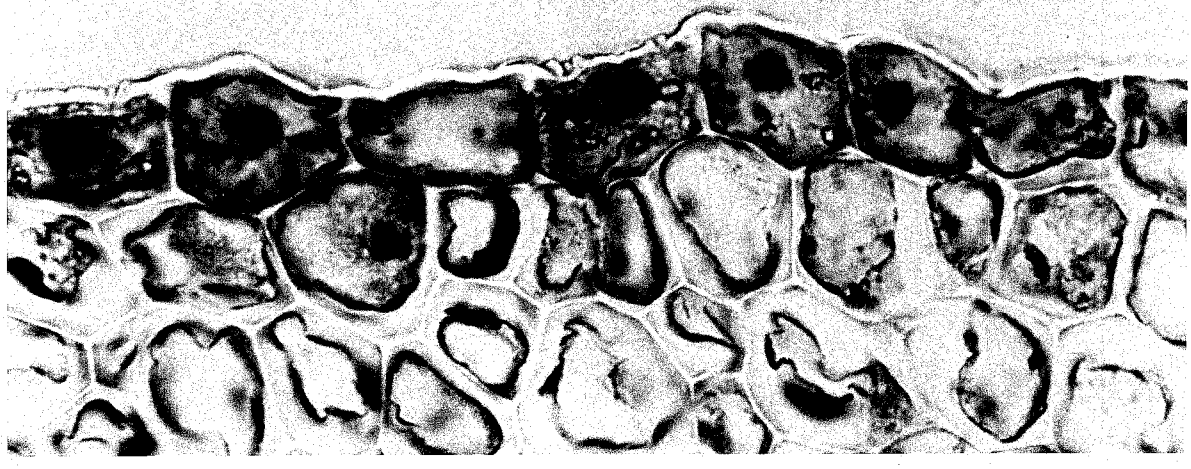
Sample Group	x Leaf Length (mm)	x Leaf Width (mm)	Leaf Thickness			Upper Epidermis Cuticle Thickness	
			a	Midrib	c	a	b (mm)
1.	17	10.25	0.2	0.72	0.2	0.0008	0.003
2.	28.30	16.25	0.22	0.72	0.26	0.0012	0.003
3.	47.40	29.75	0.28	0.86	0.32	0.004	0.005
4.	55.20	37.50	0.32	0.86	0.30	0.004	0.005
5.	74.50	53.00	0.30	1.02	0.34	0.003	0.005
6.	61.33	40.00	0.34	1.0	0.36	0.006	0.01

Fig 25

Cuticle developement with increased leaf size. (mag x 450)



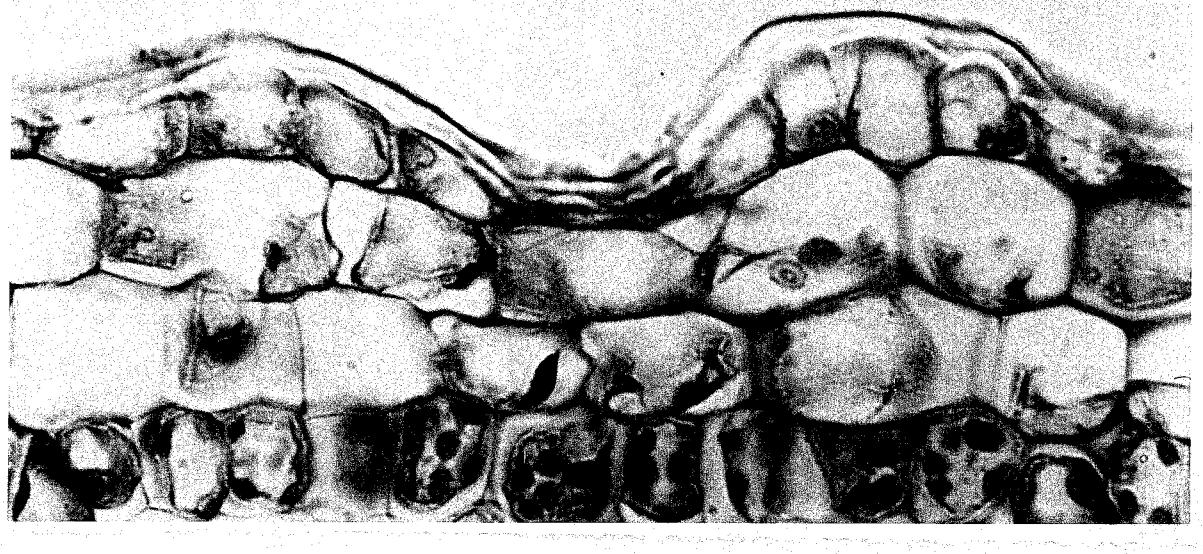
GROUP 1 Table 2b.



GROUP 2

Cuticle development with increased leaf size.

GROUP 3.



GROUP 5

Cuticle development with increased leaf size.

GROUP 6.

Comparisons of new leaf cuticle thickness at the midrib with cuticle thickness at the midrib for the previous seasons growth gave a significant difference in values even at 1% level of significance (t - 5.5). Thus new leaf tissue offers less resistance to penetrating organisms, hence they are more prone to attack compared with older leaves, for which the defence barrier is more developed.

Early in the investigation it was hypothesized that leaf cuticle thickness may influence the rate of infestation of individual trees by P. ilicis, since thinner leaves are more easily penetrated by the ovipositor. This hypothesis was investigated, but since leaf size and thickness have been shown to be correlated, leaves used in comparisons between trees were of a similar size to reduce error. Mean leaf thickness of mined leaves were compared with the thickness of unmined leaves, (Table27). These results were related to estimates for population density of P. ilicis per tree using egg number per 100 leaves.

When leaf thickness for the two sets of leaves (Table 27), were compared using a 2 x 2 contingency test, results obtained proved that the observed differences in leaf thickness for mined and unmined leaves was insignificant. Thus the choice of leaf for oviposition and ultimately the overall rate of infestation was independant of leaf thickness.

The values for the cuticle thickness measured at three points compared with values for population density are given in Table28 for the upper epidermis only. The values obtained at a and c were bulked and mean values used.

From Table 28, trees with lowest population densities were Trees 1,2,8,11 and 13. For Tree 1,2 and 11 the values for cuticle thickness were high thus infestation would be expected to be lower in these cases since a developed cuticle will impede oviposition. For Tree 13, cuticle thickness was very low hence egg density was expected to be higher. For high population densities i.e. Trees 5,6 and 15, the values for cuticle thickness were high. In these cases following a similar argument, infestation was expected to be lower, since a thicker cuticle was assumed to impede egg laying by adult Phytomyza . When cuticle thickness and population density were tested, again using a 2 x 2 contingency test, results obtained were insignificant. Thus it would appear that the intensity of infestation is independant of cuticle thickness. However it is obvious that cuticle thickness is important in egg laying since only new growth is infested. Hence it is probable that differences in the cuticle thickness for individual leaves on the sample trees do not impede oviposition by adult leaf miners, providing the cuticle is below the critical level. All measurements concerning leaf data was carried out on infested leaves of the 1977 season, hence it would have been useful, time provided, to follow the increase in cuticle thickness of the new season's growth to determine the critical cuticle thickness preventing further egg laying. Also comparisons between population density and leaf parameters are more meaningful at the begining of the season where error between trees due to possible differences in rates of development are minimal.

Table 27.

Results for leaf thickness between mined and unmined leaves

Tree No.	$\bar{x}$ Leaf thickness of leaves with Mines (mm)			$\bar{x}$ Leaf thickness of leaves without Mines (mm)			Eggs per 100 Leaves
	a	b	c	a	b	c	
2.	0.55	1.32	0.50	0.47	1.28	0.46	10.77
3.	0.43	1.12	0.45	0.43	1.07	0.42	25.70
4.	0.46	1.07	0.41	0.41	1.10	0.41	18.58
5.	0.39	1.10	0.41	0.39	0.96	0.4	33.24
6.	0.39	1.24	0.41	0.43	1.17	0.43	32.06
7.	0.36	1.13	0.41	0.45	1.15	0.44	23.70
8.	0.37	1.01	0.37	0.37	1.01	0.33	14.08
9.	0.51	1.12	0.52	0.46	1.03	0.44	18.71
10.	0.35	0.97	0.35	0.42	1.08	0.43	31.37
11.	0.49	1.0	0.42	0.49	1.10	0.49	13.06
12.	0.42	1.08	0.46	0.40	1.05	0.40	30.93
13.	0.43	1.03	0.45	0.37	0.93	0.41	15.83
14.	0.39	0.96	0.41	0.40	0.95	0.4	28.21
15.	0.39	0.98	0.38	0.38	0.95	0.32	40.21
16.	0.41	1.06	0.39	0.35	1.00	0.38	20.89

Table 28.

Results for cuticle thickness measured at the midrib for Trees 1 - 16

Eggs per 100 leaves	$\bar{x}$ Cuticle thickness b	a	Tree No.
13.42	0.015	0.008	1.
10.77	0.013	0.008	2.
25.70	0.01	0.006	3.
18.58	0.013	0.01	4.
33.24	0.015	0.008	5.
32.06	0.015	0.009	6.
23.70	0.008	0.006	7.
14.08	0.011	0.008	8.
18.71	0.013	0.008	9.
31.37	0.013	0.008	10.
13.06	0.013	0.008	11.
30.93	0.01	0.008	12.
15.83	0.009	0.006	13.
28.21	0.015	0.01	14.
40.21	0.01	0.006	15.
20.89	0.01	0.008	16.

4.5. Life-history of Phytomyza ilicis and its major parasites.

A diagram indicating the life-history of Phytomyza and its parasite is given by Lewis and Taylor (1974). The figure indicates the various developmental stages present in each month for the U.K. To summarize the egg stage for P. ilicis was recorded from early to late June. The larval stages spanned from the middle of June until the end of March and overlapped with the beginning of the prepupal and pupal stages. Adult emergence spanned from late May until early July. For the larval parasite Chrysocharis gemma, the egg stage spanned from late February until early April, again overlapping with the larval stage which spanned from early March until early May. The prepupal and pupal stages were observed from the middle of April until late June. The adult stage was recorded from June. For Chrysocharis syma, a pupal parasite, the egg stage was recorded from late February until early April. Larvae were observed from early March until late May. This stage was overlapped by prepupal and pupal stages which were recorded from late May until the middle of June. Adult C. syma emerged from mid June onwards.

For Sphegigaster flavicornis also a pupal parasite, egg laying spanned from early to late April. The larval stage was recorded from late April until the end of May. Prepupal and pupal stages spanned from mid May until late July. Adult S. flavicornis were recorded from early June.

Thus the egg stages for Chrysocharis gemma and C. syma were approximately in phase, that for Sphegigaster flavicornis being out of phase by one month. P. ilicis was observed to have the longest larval stage, the shortest spanning approximately one month was observed for S. flavicornis. The shortest prepupal to pupal stage was observed for C. syma, again spanning approximately one month.

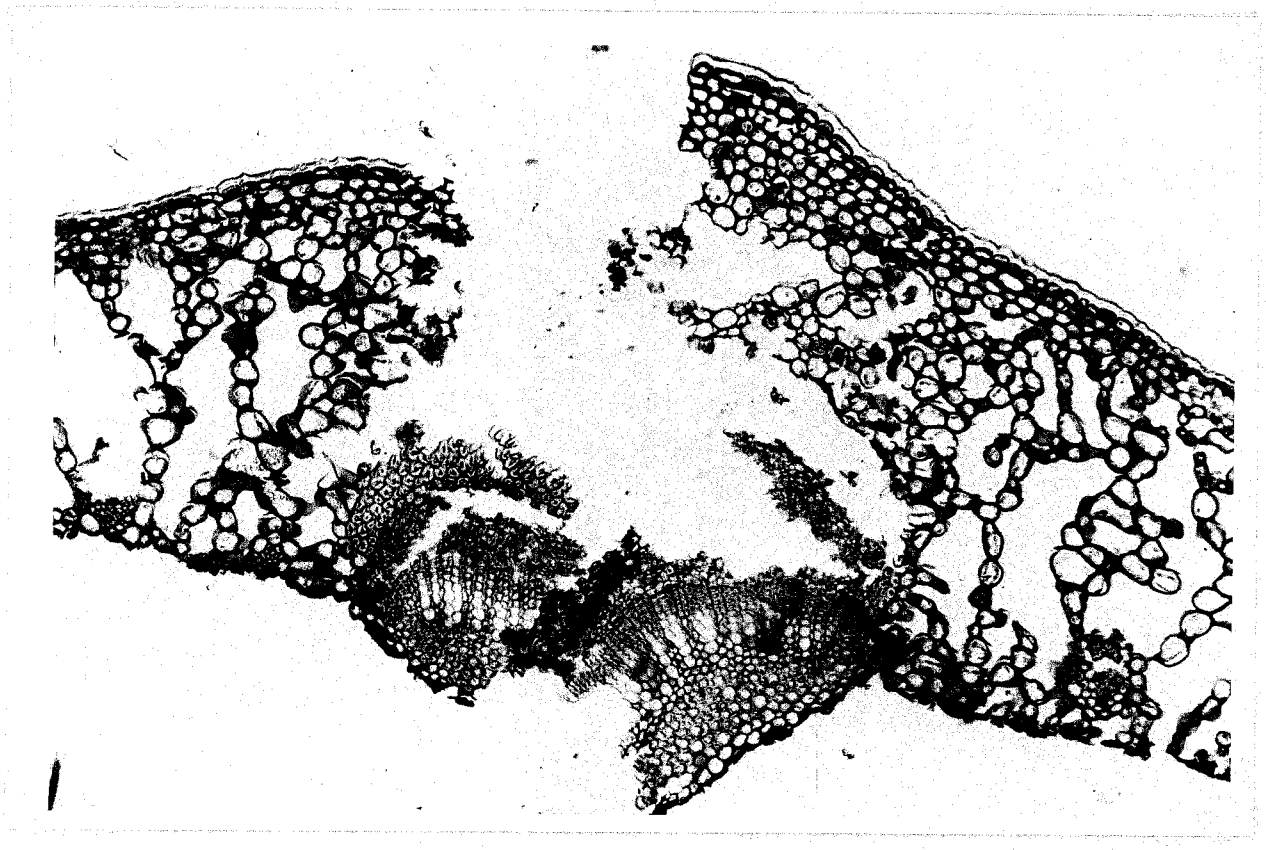
From the beginning of this sampling program April 24th, 1978, P. ilicis was at the pupal stage. The pupae were pale orange, between 2.6 to 2.9 mm, and body segmentation within the pupae was in the early stages of development. As the pupae developed they darkened to a brown colouration. Mature larvae were also observed, but these were always shrivelled and dead. The first recordings of adult emergence was 2nd June, 1978, where 7 from 49 pupae had emerged. By the time sampling was completed 17th July, 1978, 45 from 118 adult Phytomyza had emerged. C. gemma was observed at the prepupal to adult stages, while C. syma was observed from mature larvae to adult stages. For Sphegigaster flavicornis, being data obtained from mine examinations, the species was observed from the mature larval to prepupal stages. However a few adults were extracted from suction-traps but were not recorded until early July.

The developmental stages for P. ilicis and its three main parasite species is given in Fig. 30-37.

Fig.26 T.S.Leaf-section taken across the mine caused by burrowing P.ilicis.larva.

(mag ×180)

a.Across the midrib.



b.Within the leaf lamina.

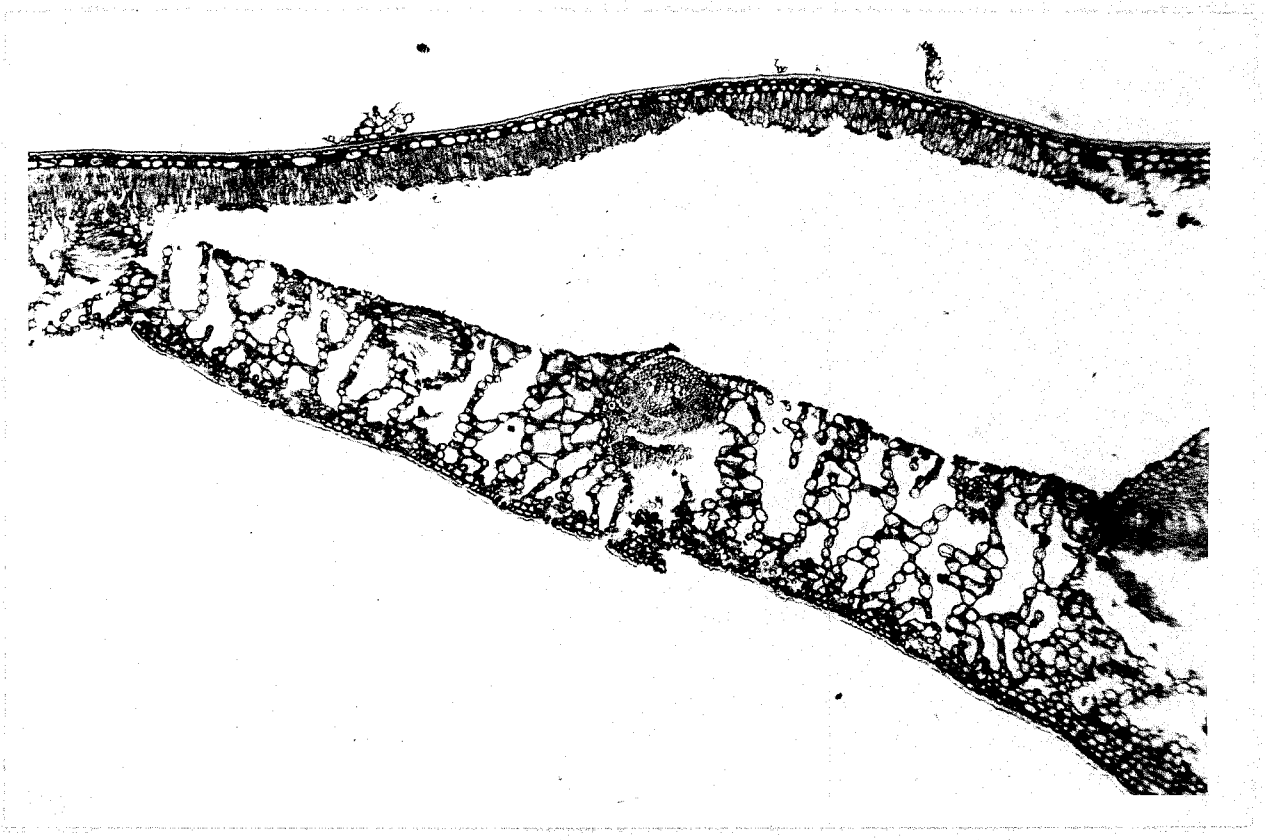


Fig 27

Hinged emergence plate over the exit hole through which Adult *P. ilicis* emerge

(mag x40)

Fig 28 Exit hole within the mine through which Adult *P. ilicis* emerge.

Fig 29

V SHAPE PECK MARK WITHIN THE MINE CAUSED BY FEEDING BIRDS.

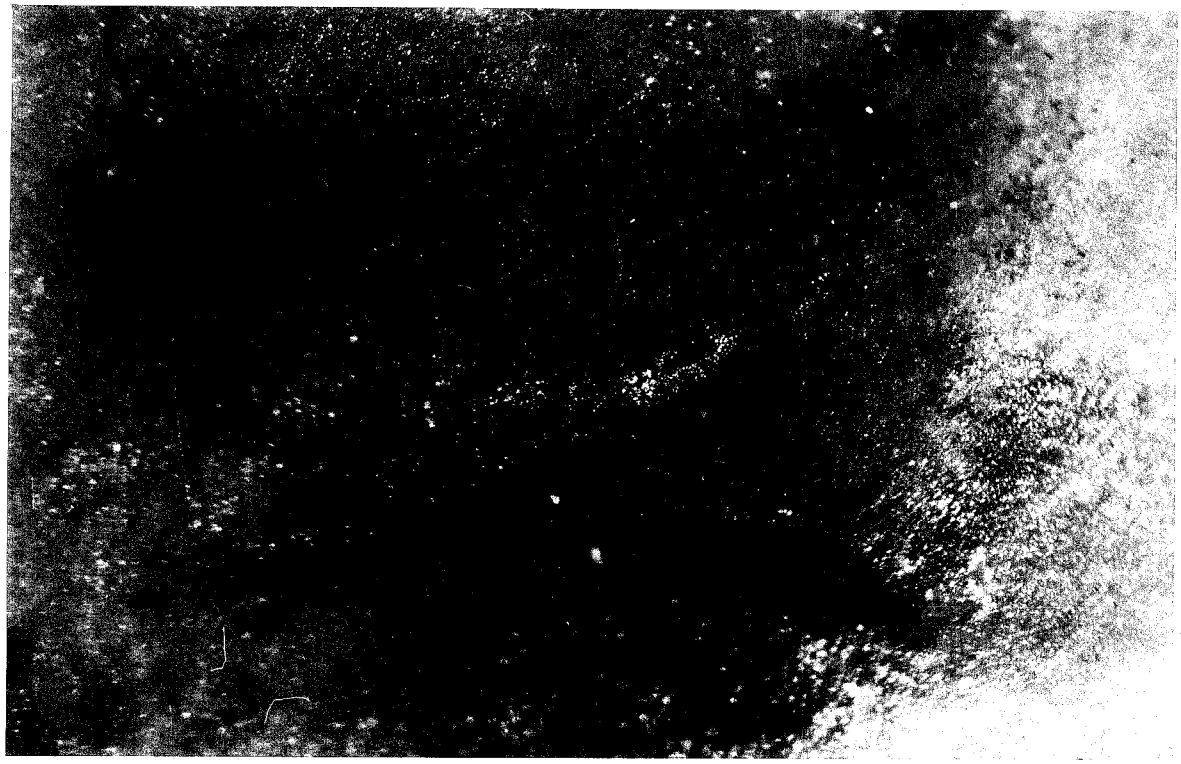
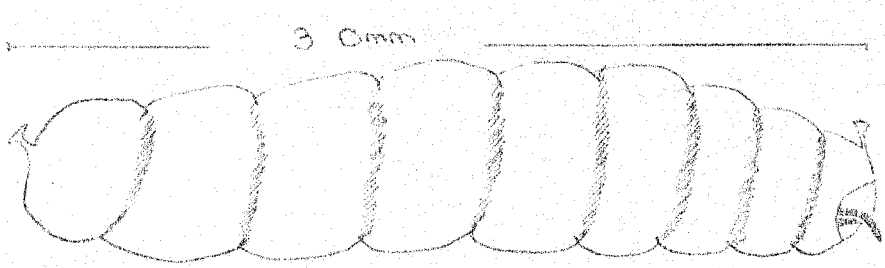
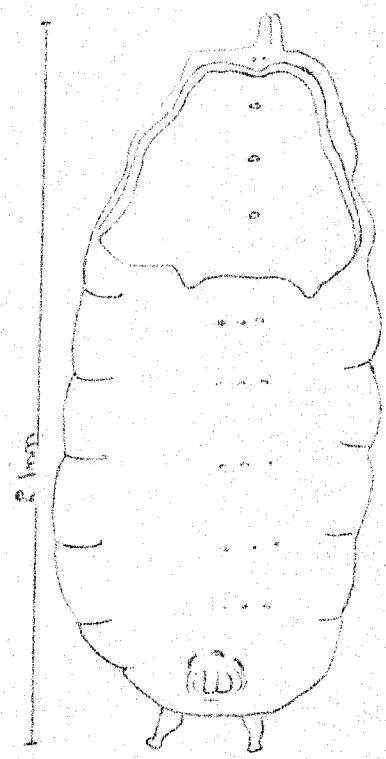


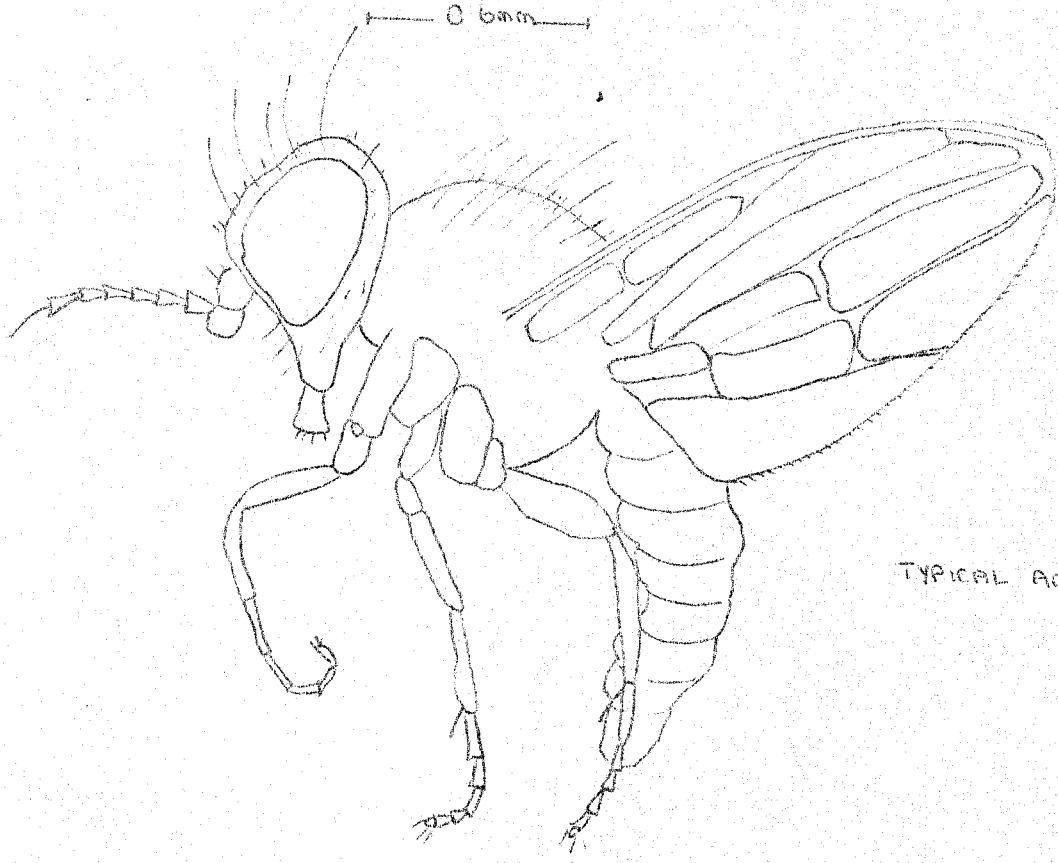
Fig 30. DEVELOPMENTAL STAGES FOR P. INICIS



P. INICIS MATURE LARVA



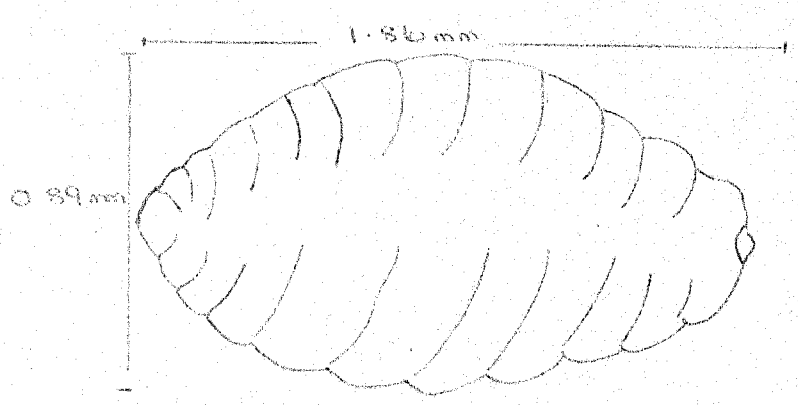
P. INICIS PUPA



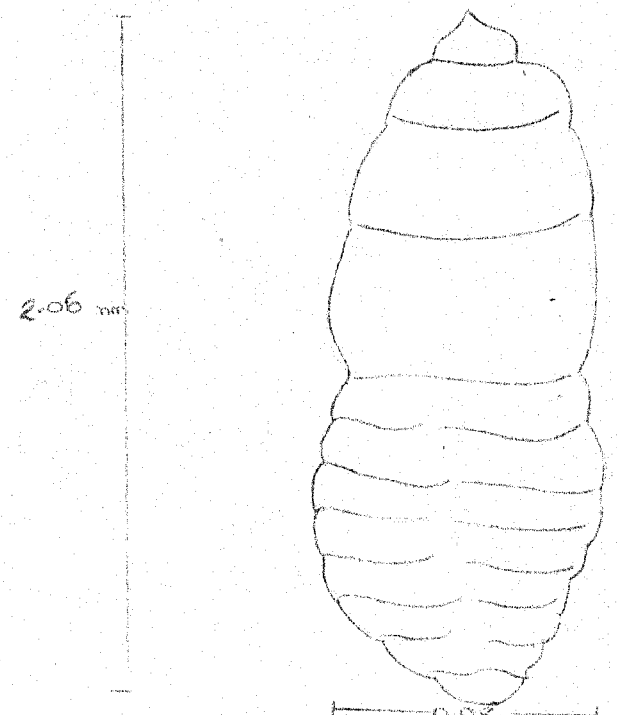
TYPICAL ADULT ARMY

Fig 31

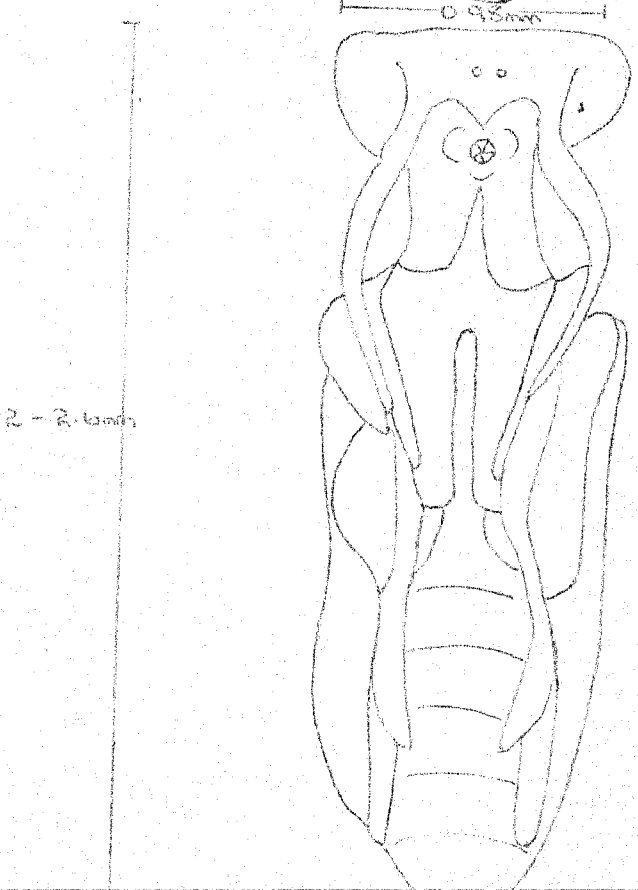
DEVELOPMENTAL STAGES FOR C. SYMA - A PUPAL PARASITE



C. SYMA MATURE LAR



C SYMA PREPUPA.



C SYMA PUPA

Fig 32.

Developmental stages for C. syma / C. gemma.

(mag x 100)

a) PREPUPA

b) Pupa

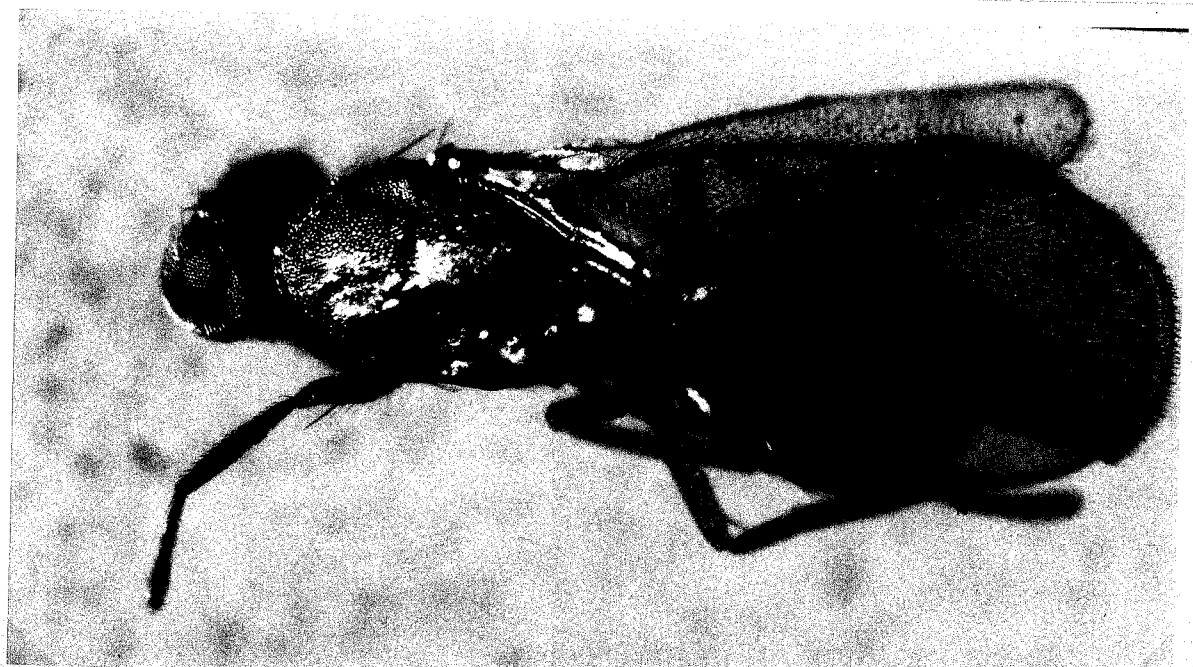
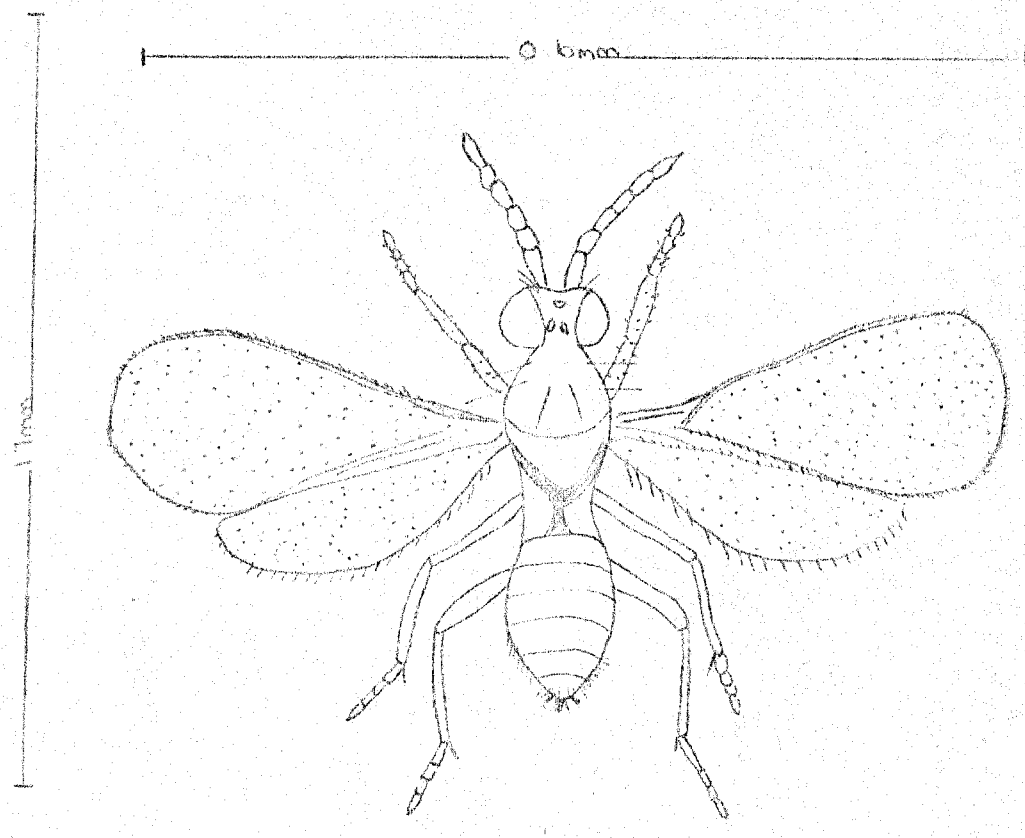


Fig 33

ADULT C. SYMA. / C. GEMMA.



ADULT S. FLAVICORNIS.

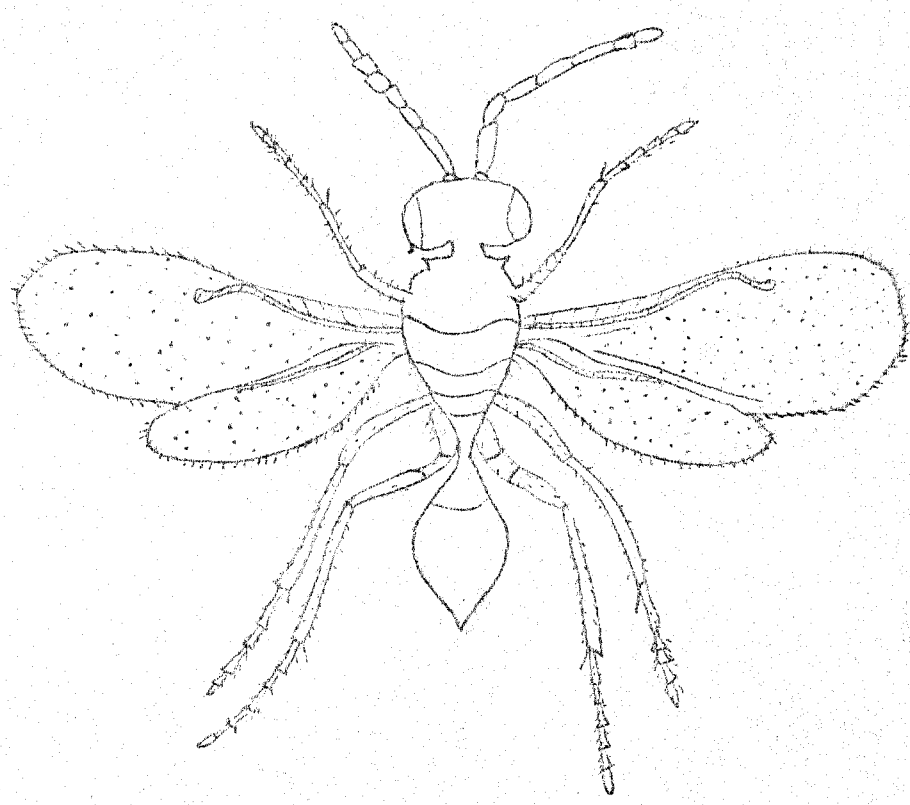
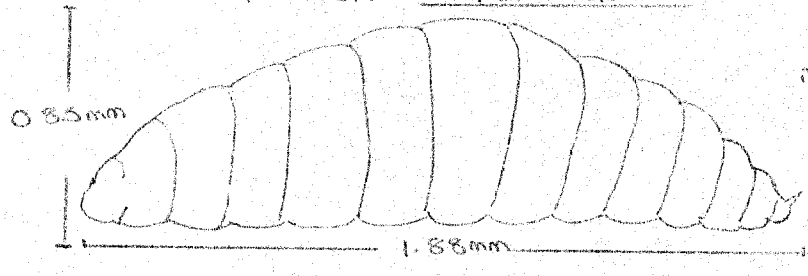
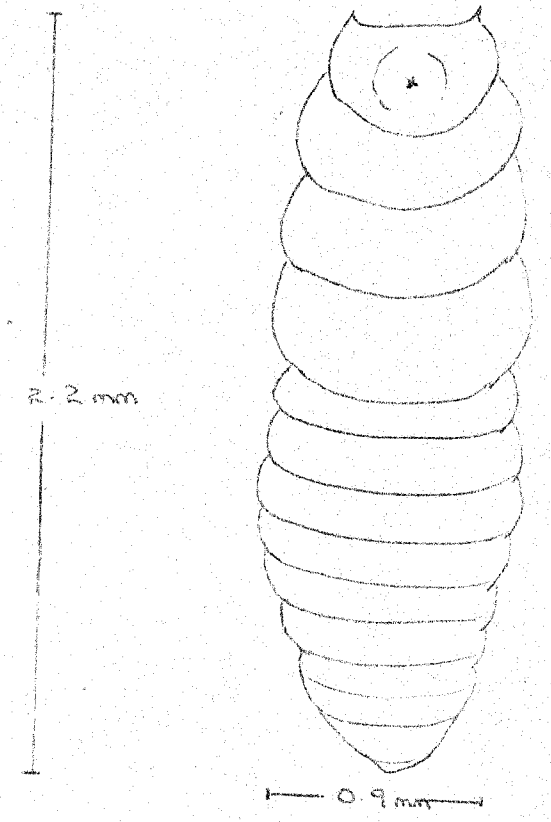


Fig 34.

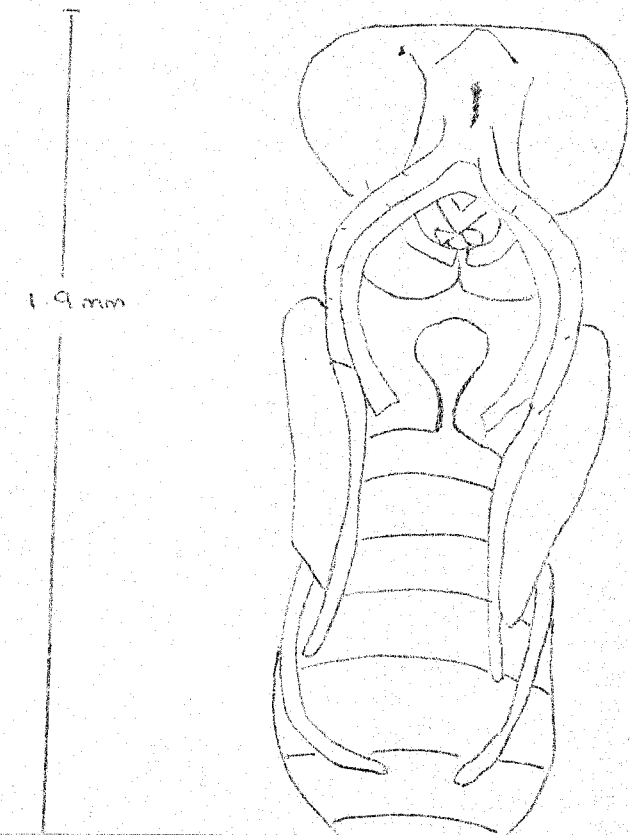
DEVELOPMENTAL STAGES FOR S. FLAVICORNIS.



MATURE LAR



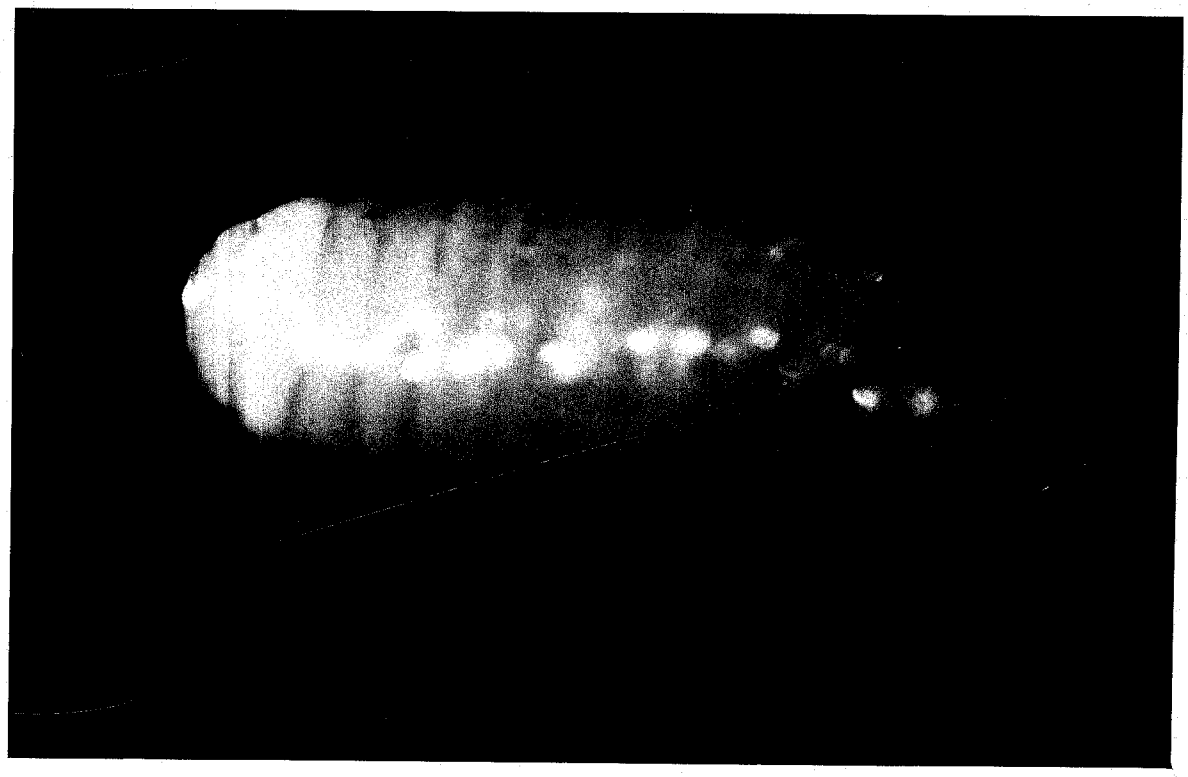
PREPUPA



PUPA - (NOT OBSERVED
LEWIS AND TAYLOR (19

Fig 35.

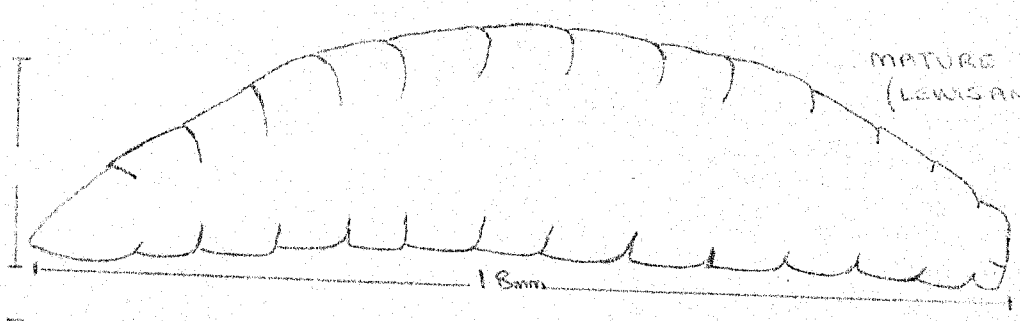
S. FLAVICORNIS MATURE LARVA (mag x64)



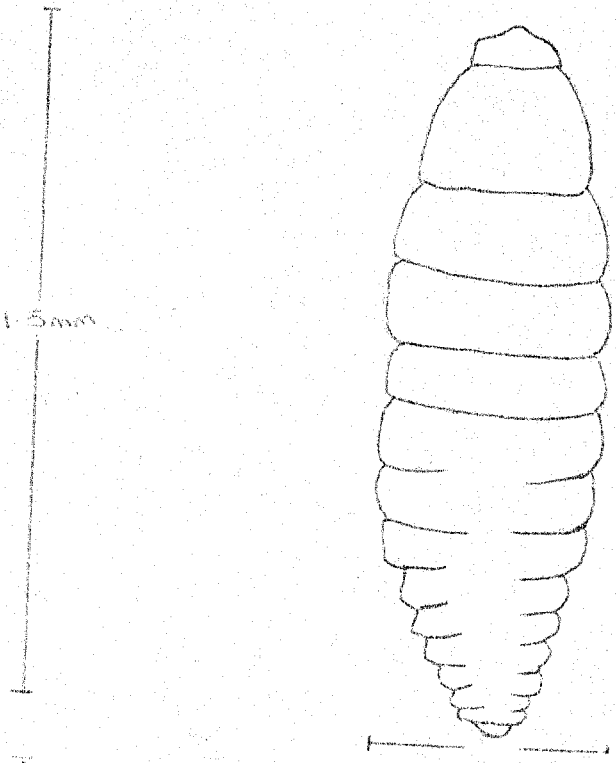
ADULT S. FLAVICORNIS

Fig 36.

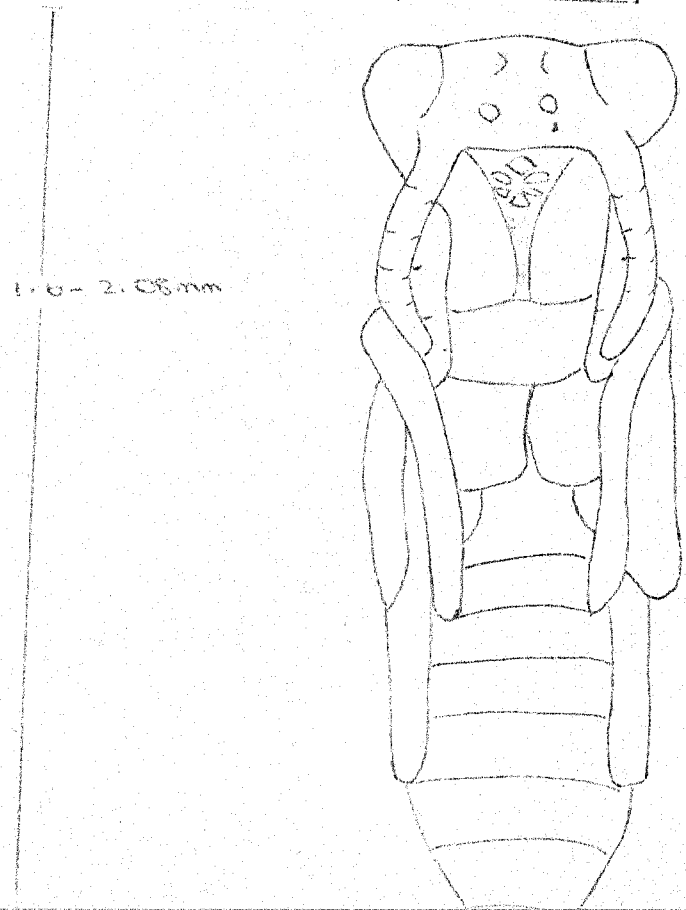
DEVELOPMENTAL STAGES FOR C. GEMMA



MATURE LARVA (LEWIS AND TAYL 1974



PREPUPA (LEWIS AND TAY 1974)



PUPA.

Fig 37

ADULT CHRYSOCHARIS GEMMA.

(mag x64)

For Tree 2 examined on 15.5.78, C. syma was at the mature larval stage although a few prepupae had been recorded.

Tree 3 was examined on 16.5.78, 8.6.78, 14.6.78 and finally 22.6.78. From 16.5.78 C. gemma was at the pupal stage. Prepupae of S. flavicornis were recorded but from later samples taken 8.6.78. By 14.6.78, P. ilicis had begun to emerge, together with C. gemma , but a larger proportion of both P. ilicis pupae and the larval parasite pupae still remained within the mine. C. syma was recorded from the mature larval stage onwards, however the former was dominant in samples. 3 adult Phytomyza with the characteristic black head and brown segmented abdomen were obtained from 22.6.78 samples. 5 adult C. syma were observed from the same sample the thorax was dark fluorescent green, the abdomen being black and flat. Adult C. gemma were also extracted but were easy to distinguish because of thier red-brown fluorescent colour and the thorax and abdomen were smaller and thinner, i.e. body-size 1.7 x 0.6 mm.

Tree 6 was also sampled on 14.6.78. 13% of C. gemma had emerged. C. syma pupal stage was dominant in samples although mature larvae and prepupae were also recorded. Thus a variation in the rate of development between trees was found.

Tree 10 was sampled on 29.6.78. P. ilicis within the pupae was well developed. For the sample 0 - 50 cms, 21% of P. ilicis had emerged. C. gemma and C. syma were observed at pupal stages. S. flavicornis was recorded at the mature larval stage but these generally were unhealthy.

Tree 15 was sampled on 13.7.78. 3 adult C. syma were observed to emerge from the pupae, characterized by their body shape and fluorescent green colouration on the thorax. 2 C. gemma adults were observed, again the thorax was thinner and fluorescent brown in comparison with C. syma. 86% of C. gemma had emerged. C. syma was at the pupal stages and S. flavicornis at the prepupal stage.

Measurements of body length and width are given in Table 29, 30, 31. Mean measurements were taken from larvae and prepupae extracted from all trees. Enough samples were not collected to make comparisons in size between all trees, and those which had been collected had been stored in alcohol before measurement causing distorsion.

From Table 29 there is little variation in the size of P. ilicis pupae with sample tree. It was thought that possible differences in the level of nutrition between trees may influence the size and rate of development of pupae. It was observed that difference in the stage of development existed between trees, but this was more likely to be associated with the time of egg laying than nutritional content of the leaves, i.e. Tree 8, was an unhealthy tree. It was heavily shaded and grew on comparitively unfertile ground. The cuticle in comparison with other trees was thinner and the leaves were dull. However pupae extracted from infested leaves measured 2.94 x 1.24 mm, thus were larger than pupae extracted from more healthy hosts. However the size of C. gemma, which attacks P. ilicis larvae was small in comparison to other trees. Mine size was correlated with C. gemma pupal size, and a significant r value of 0.56 was obtained. The size of the mine is related to the feeding activity of P. ilicis larva, maximum mine size occuring in March when mature larvae are present.

Table ..29.

Tree No.	P. ilicis	Pupa.
	$\bar{x}$ Length	Width
1.	2.9	1.23
2.	2.87	1.21
3.	2.82	0.95
4.	2.88	1.20
5.	2.89	1.24
6.	2.75	1.28
7.	2.94	1.20
8.	2.94	1.20
9.	2.9	1.11
10.	2.85	1.20
11.	2.85	1.13
12.	2.65	1.20
13.	2.73	1.22
14.	2.66	1.10
15.	2.82	1.24
16.	2.66	1.10

Variation in the size of P. ilicis pupa with Sample Tree

Table 30..

	C. gemma	Pupae
Tree No.	Length	Width
1.	-	-
2.	-	-
3.	2.01	0.62
4.	2.05	0.73
5.	2.08	0.75
6.	2.01	0.70
7.	2.07	0.68
8.	1.63	0.55
9.	2.01	0.69
10.	1.95	0.68
11.	2.11	0.72
12.	2.03	0.67
13.	2.09	0.68
14.	-	-
15.	1.6	0.35
16.	2.00	0.6

Variation in the size of C. gemma with Sample Tree

Table 31..

	C. syma	Pupae
Tree No.	Length	Width
1.	2.1	0.8
2.	2.0	0.7
3.	2.06	0.8
4.	2.0	0.6
5.	2.2	0.7
6.	2.16	0.88
7.	2.19	0.95
8.	Not Measured	Not Measured
9.	2.5	1.11
10.	2.2	0.89
11.	2.1	0.96
12.	2.16	0.74
13.	2.1	0.8
14.	2.25	0.85
15.	2.2	0.8
16.	2.66	0.3

Variation in the size of C syma with Sample Tree

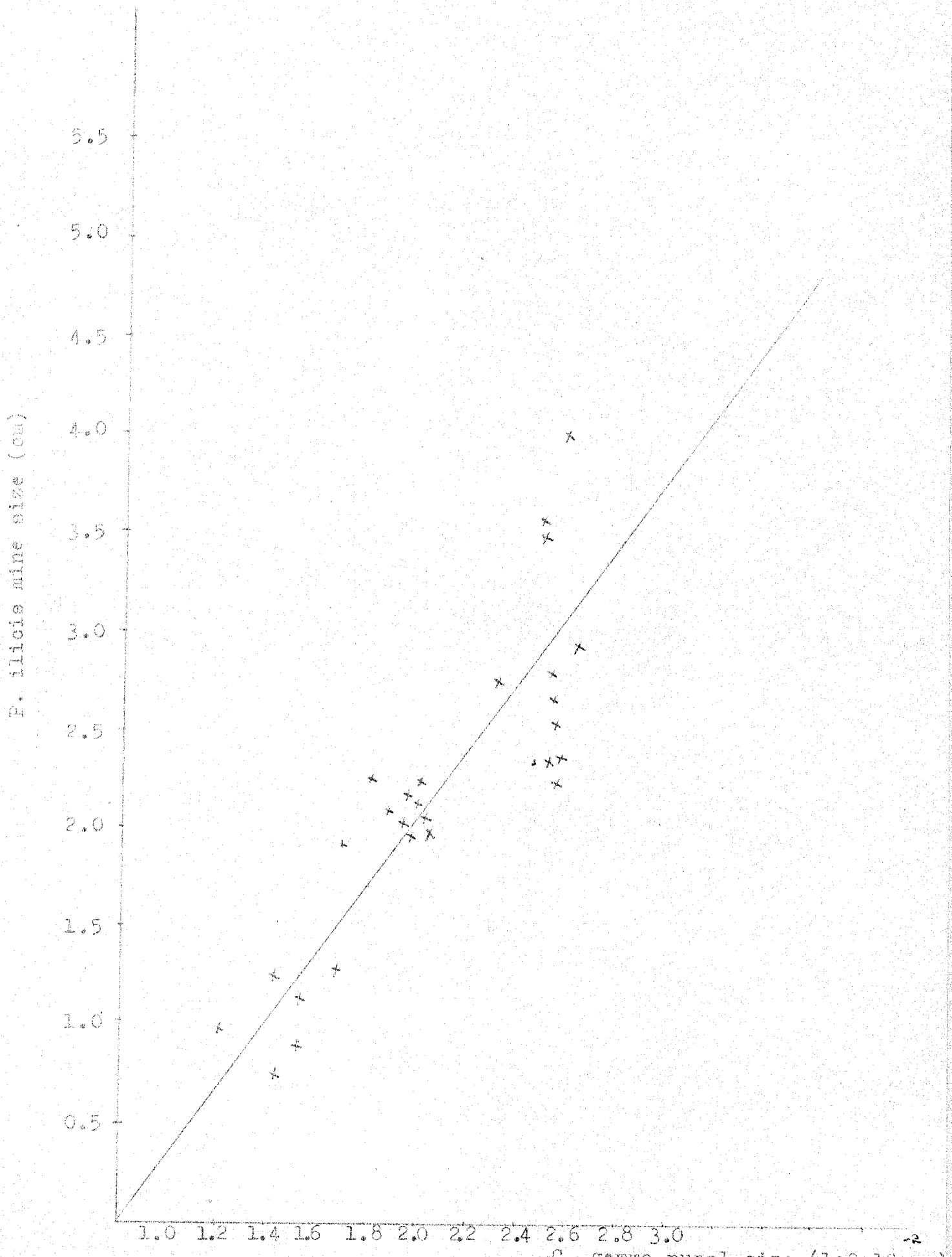
Thus it would appear that the size and state of health of P. ilicis larvae influenced the final size of C. gemma pupae. Thus C. gemma from undersized P. ilicis larvae tend to pupate at a lower overall body size compared with those emerging from larger miner larvae. This would suggest timing of pupation rather than final body size is more important. However further work is required before a firm conclusion can be made.

Table ..32.

Mine Size. cm.	C. gemma. mm. Body Length x Body Width
1.95 x 2.6	2.5 x 1.0
1.95 x 0.65	1.9 x 0.7
1.3 x 0.975	1.6 x 0.5
2.28 x 1.30	1.7 x 0.6
2.76 x 2.28	1.9 x 0.7
2.28 x 1.30	2.0 x 0.7
2.93 x 1.95	2.6 x 1.0
5.9 x 1.30	2.4 x 0.9
0.81 x 0.65	1.4 x 0.3
2.28 x 0.65	2.5 x 0.9
2.11 x 1.79	1.9 x 0.7
2.2 x 0.49	2.0 x 0.7
3.58 x 2.93	2.5 x 1.0
3.25 x 0.65	2.5 x 0.9
2.28 x 1.63	2.0 x 0.75
0.975 x 0.65	1.2 x 0.4
2.28 x 0.975	2.0 x 0.75
2.11 x 1.79	1.9 x 0.6
2.61 x 1.3	2.5 x 1.0
1.79 x 2.925	1.7 x 0.6
1.3 x 0.975	1.6 x 0.5
1.95 x 0.813	1.9 x 0.5
0.975 x 0.975	1.5 x 0.6
2.93 x 2.61	2.5 x 0.9
1.95 x 1.3	1.9 x 0.7
3.9 x 1.3	2.6 x 1.0
1.79 x 2.925	2.5 x 1.0
1.3 x 1.3	1.4 x 0.4
2.925 x 1.3	2.5 x 1.0
2.76 x 1.3	2.3 x 0.9
1.95 x 1.79	1.9 x 0.6
1.95 x 1.63	1.9 x 0.5
1.14 x 0.813	1.5 x 0.5

Fig. 38.

F. ilicis Mine length plotted against pupal size for C. gemma.



5. Discussion.

A variation in the time of emergence for adult Phytomyza ilicis was observed when compared with accounts given by Miall and Taylor (1907), and Downes (1931), although the general pattern of the life-cycle was fairly consistent with thier account.

In accordance with these authors, adult emergence was maximum during mid June, and flies were observed to be plentiful amongst holly bushes. This however, was never the case during this investigation. Suction-traps positioned within a clump of 3 holly trees failed to collect any adult flies, although a few adults of the pupal parasite, Sphegigaster flavicornis were collected. The flight pattern of adult flies may have influenced whether they were collected in traps, since both adult Chrysocharis gemma and C. syma were observed to hop like flies between leaves when interfered with (Cameron 1939). Thus it is possible that flies avoided these areas above the traps from which samples were extracted. The behaviour of adult P. ilicis was not recorded, hence it is impossible to determine accurately why these adults were absent in samples.

For this investigation P. ilicis adults were first recorded from 2nd June, 1978. A s ample of 49 pupae was collected from which 7 adults had already emerged. However maximum emergence was observed during early July where 45 from a sample of 118 pupae had emerged. Body measurements for adult P. ilicis were 2.1 mm for body length and 0.6 mm for body width. The head was black and the abdomen, consisting of 9 segments was brown. The halteres were white, the thorax, a dark fluorescent green/black colour. The antennae were long and segmented.

Chrysocharis gemma, a larval parasite was extracted from samples taken 16th May, 1978, where the parasite was at the pupal stage of development. The pupae were shiny jet-black and were easily identified since they lay free within the mine. Wing and leg rudiments, eyes, mouth appendages, in addition to thorax and abdominal segmentation were clearly marked. Body measurements gave mean values for body length of between 1.6 - 2.08 mm and 0.35 - 0.75 for body width. Cameron (1939), quotes values of 1.75 - 2.60 mm for body length, and 0.65 - 1.04 for body width. Adult C. gemma were first recorded from samples taken on 14.6.78, but most had emerged by 13.7.78. Adult C. gemma were reddish brown in colour which fluoresced under the microscope. The thorax and abdomen were very much thinner than those of C. syma, hence could be distinguished easily. Miall and Taylor (1907), observed that the peak of emergence for this species was the middle of June, although my results suggested peak emergence to be later for the Durham area.

Chrysocharis syma, a pupal parasite, was recorded at the mature larval stage from samples taken on 15.5.78. The prepupal stage was recorded from late May onwards. C. syma larvae were fat and fusiform in shape, being a greyish white in colour with a central mass of black faeces. Measurements of the mature larvae were inaccurate since the samples had been stored for some time in alcohol prior to measurement. The mean value for body length and width for the mature larvae was 1.86 mm x 0.89 mm. Cameron (1939) quotes values of 2.14 mm x 0.97 mm for body length and width respectively. The prepupal stage was distinctive. This is characterized by broad thoraic segments which are both smooth

and shiny, differing from the prepupae of Sphegigaster for which the segments are narrower and more contracted with deeply rounded sides. The abdomen for C. syma is more bulbous and consists of 10 segments. However identification was not as easy as suggested since the alcohol preservative again caused distorsion in the samples. Body measurements for prepupae were 2.06 mm x 0.98 mm for body length and width respectively. Values quoted by Cameron are 2.2 mm x 0.84 mm, but he observed that prepupae tended to contract before entering the pupal stage so that the final body measurements were 1.70 x 0.80 mm. C. syma pupae were observed from 14.6.78. These were black and shiny and can be distinguished from other pupal parasites of P. ilicis by the bow-like shape of the body from the wing angle to the posterior segment of the abdomen. Body measurements for C. syma ranged between 2.0 - 2.6 mm for length x 0.3 - 1.11 for width, hence were larger than those recorded by Cameron (1939). Adult C. syma were recorded from 13.7.78, thus emergence was later than that given by Cameron, where adults were observed from mid June onwards.

Sphegigaster flavicornis was recorded at the prepupal stage from 8.6.78, although mature larvae were also present in the samples. Mature larvae were white and contained large brown internal faecal masses within. Measurements of body length were 1.32 mm to 2.2 for body width, compared with 2.1 - 2.3 mm x 0.92 - 0.97 mm for values quoted by Cameron. The prepupae of Sphegigaster are characterized by the antennae of the old larval head, and as mentioned earlier the thoracic segments are more rounded. The prepupal body length was 2.2 mm and that for body width 0.9 mm, again varying from values of 1.95 mm x 0.75 mm given by Cameron (1939). The parasite was at the prepupal stage up to 17.7.78 when the final sample was taken. Cameron (1939), observed that adult emergence occured about the middle of June, the peak being in the latter half of the month. Thus results obtained again differ. Climatic factors probably caused the delay in development observed, since physical factors were far more extreme compared to Southern areas sampled by Cameron, and many species of insects have been observed to undergo a period of diapause associated with extreme weather conditions.

The main problem with the investigation was the sampling method used. The method finally adopted was random and was ultimately dictated by the growth manner of the trees sampled. For the taller trees it was difficult to sample accurately at the crown and leaves per twig were sparse at these levels anyway. Initially it was decided to compare the rates of infestation within the tree i.e. height and aspect variation by using equal numbers of twigs. However problems were involved with the actual comparisons since the number of leaves per twig varied considerably (3.1) . Although values for egg and mine density were expressed per 100 leaves sampled, results were both easier to handle and more accurate when equal numbers of leaves were sampled. For Tree 1, when 42 twigs were sampled per side, the variation in leaf number was 467 for the East - 1,115 for the West side. Using the smaller but constant sample size similar trends in the level of infestati were obtained, although the overlap between West and North, and East and Southern aspects were more pronounced (Tables 6a and 6b.

by comparisons of values for infestation rates between trees based on 100 and between 325 - 4,300 leaves(Tables 4 and5), the increase in accuracy of estimates is given by the reduced confidence-intervals.

Stokes (1969), decided the optimum sample size was 750 leaves per tree. 300 - 1,000 leaves were considered as the minimal sample size in this investigation as mortality factors affecting I. ilicis pupae were considered. However for most trees the sample size was greater, and again was influenced by the size of the tree (i.e. 325 - 4300leaves).

Morris (1955), distinguishes between various types of population estimates, i.e. absolute estimates, relative estimates and population indices. Absolute estimates are expressed as the number of animals per unit area. Relative estimates are expressed in unknown units and always depend on factors other than population size. Population indices are estimates of the size of the population base on the activity of animals, i.e. the amount of faecal matter produced. Hence for this investigation absolute estimates are more useful. Morris further divides absolute estimates into absolute population i.e. expressed as a number of animals per unit of ground population intensity - the number of animals per unit of substratum and a basic population as a number per unit of intermediate size chosen for convenience. Thus when population size is estimated it usually involves the calculation of the population intensity, which is then converted to an absolute population using information about the size and spatial distribution of the host plants.

During the study, population estimates for P. ilicis were based on total egg number expressed per 100 leaves, since it was impossible to obtain an absolute population as no information on the number of leaves per tree was recorded. Thus a basic population estimate was finally obtained for all trees.

No significant difference was observed in the level of infestation, i.e. total number of leaves with mines expressed as a % of total leaf number, although both total egg number and total mine number varied significantly between trees (4.1.2). Trees least affected were trees 1,2,8 and 13. Trees 1 and 2 were most isolated, however when the final population density and degree of isolation of trees from their neighbouring holly trees was investigated, no significant correlation was found. Thus spatial distribution did not appear to influence the density of leaf miners although the more isolated Trees 1 and 2 were lower in total egg density per 100 leaves. No significant difference in population density with regard to position within Hollingside Wood was found despite observed differences discussed in section 4.1.2. Both aspect and height influence the density of total mine and egg numbers (4.1.3). Maximum egg numbers were observed between 100 - 150 cm height interval. Total numbers recorded for egg density were considerably reduced at heights above 300 cms. The variation in population density with aspect was not so conclusive. Generally higher egg counts per 100 leaves were observed for the East side of the tree. This may have been because Eastern areas were more sheltered in terms of wind exposure since they faced into the wood, although information on climatic factors is required before any conclusions can be drawn.

Since East sides were overshadowed to a greater extent by neighbouring trees, fewer leaves per twig were found, hence it is possible that adult P. ilicis may have laid eggs on leaves in the normal manner, but as fewer host leaves were present, this restricted their oviposition to a smaller area, thus used more of the leaves on a given twig for oviposition rather than spend a great deal of time searching between leaves. Thus when the samples were collected the population density results obtained would be greater since these results are expressed as egg number per 100 leaves. Hence whether this difference in aspect is real or due to lower leaf number on the East facing side is open to question. It is possible that a larger proportion of young leaves were available at the time of egg laying on the East side, although generally this is unlikely. The biochemical content of leaves may also influence preference for oviposition sites. Different parts of plants and of the same plant under different conditions and at different seasons were observed to vary in their biochemical composition (Southwood 1973). Thus it is likely that the changes observed in the feeding preference of any insect, development rate, fecundity and survival is associated with changes in the growing conditions of hosts plants.

Stokes (1969), found no variation in infestation with height or aspects.

Variation in population density with host plants within the same area has been observed for many species of phytophagous insects. Varley and Gradwell (1965), working with Operophtera brumata L., concluded that variations in the egg population sizes of the winter moth on different oaks within the same area was due to differences in the time of bud break between trees. Each tree began leaf expansion on different dates in each season, according to weather conditions, but it was found that the order of bud break among a group of oaks investigated over several seasons was constant individual trees following each other in a regular sequence from year to year. Askew (1961), gave a similar explanation for differences between individual young oaks, in the populations of galls formed by Neuroterus sp. The time of leaf expansion and ultimately cuticle thickening is important, since the cuticle is the main defence barrier preventing miner attack. This has been indicated in section (4.4). Young leaves could avoid attack either by expanding early in the season thus the cuticle may develops so as to act as a more effective barrier. In addition leaves expanding later in the season avoid the peak times for adult P. ilicis emergence, hence are less liable to be infested. It has not been determined whether leaf expansion varied between trees in the manner given by Varley and Gradwell (1965), although it seems feasible that variation in population density may be attributed to the number of host leaves available for attack.

Stokes (1969), suggested that the degree of spatial isolation of individual holly trees was a more important factor. For the Ancient Camp area, estimates of population density varied a great deal between sample trees i.e. 5.2 eggs per 100 leaves - 118.8 eggs per 100 leaves. He found trees with lowest P. ilicis densities were relatively isolated separated from other holly trees by 10 metres or more.

Highest egg densities were recorded from trees forming part of a clump of closely grouped trees. He suggested that more isolated trees are likely to be reinfected by females emerging from puparia derived from eggs laid in the leaves of the same tree in the previous year, however within a clump of trees, eggs may also be laid by females originating from neighbouring holly trees. Thus clumped trees will have higher values for total egg density since they are exposed to egg laying over a longer period.

In this investigation, the variation in total egg number per 100 leaves between trees was not as marked. Recorded values ranged from 16.77 - 48.61, and no significant difference between population estimates and spatial isolation were observed. This may have been because overall estimates for population density were so low.

Population density is estimated from egg numbers per 100 leaves. Thus it is possible that leaves of some of the trees are more liable to repeated attack by adult miners, hence will carry more eggs per leaf compared to other trees (Table 17).

The variation in egg number per leaf was high. For the majority of cases only one egg was laid per leaf. Increasing the number laid per leaf tended to increase egg mortality, thus reducing the numbers surviving per leaf to produce mines (4.2). The reasons why more than one egg is laid per leaf are unclear, especially as visibility is reduced with increased egg density. One explanation is that an individual adult after laying an egg in the midrib flies off to another leaf. A second individual may approach the already infested leaf and lay a second egg and so forth. However under these circumstances it would be more beneficial for the first adult to leave some type of chemical warning, i.e. pheromone, on the leaf surface as an indication that an egg had been laid. This would reduce competition for both food and space should both larvae develop. By avoiding laying more than one egg per leaf in theory, the adult increases the survival of all eggs and ensures that the holly does not become damaged to such an extent that would reduce the survival of future populations. In several cases where one egg per leaf was laid, small mines devoid of contents were found. Death of first instar larvae may have been due to bacterial disease but actual causes are undetermined. Hence it is equally likely that an individual may lay more than one egg per leaf to ensure the survival of at least one egg. Where two mines per leaf were observed it was not uncommon for one larva to develop normally and the other to become stunted and finally die, hence it is also possible that the healthy larva releases a chemical which prevents development of other larvae within the same leaf. Reasons why more than one egg is laid in some leaves and not others is also unclear, but may be related to the nutritional and biochemical content of the tissues. A final explanation could be that the adult P. ilicis searches for oviposition sites at random and it is just by chance that more than one egg is laid per leaf, the number having no overall significance. Cuticle thickness was not found to influence whether some leaves were infested and others not, since no significant difference between mined and unmined leaves. Hence the ultimate levels of infestation were independant of cuticle thickness.

However leaves are only susceptible to infestation when young and the cuticle is undeveloped (4.4). No difference in cuticle thickness between the lower and upper epidermis was observed. No significant difference in the number of mines found on the upper and lower surfaces of the leaf was also observed i.e 1234 mines on the upper surface compared with 1355 on the lower leaf surface.

For all trees egg distribution was clumped characterised by a variance in excess of the mean. Such clumped distribution are found frequently amongst insect populations (Southwood 1966).

Variation in % egg mortality was found between trees, but no significant difference was observed. Egg mortality incorporates failure of eggs to hatch, mortality of young larvae within the midrib, mortality whilst leaving the midrib for the lamina and finally death within the lamina before a recognisable mine has been produced. Although significant correlations between total egg number and total viable mines produced, and egg mortality per 100 leaves plotted against egg density per 100 leaves was found, the relationship between % egg mortality and total egg number was insignificant. Hence egg density influences the viability of eggs within the leaf but the relationship between egg mortality and egg density was assumed to be density-independant.

Mortality of larvae within the mines was caused by bird attack, attack by the larval parasite, Chrysocharis gemma, and by an undetermined mortality factor influencing survival in the early stages of development. Generally the major source of mortality was due to bird attack followed by parasite attack. Results between trees were significantly different. Trees 1 and 2 were unaffected by C. gemma. Reasons why the trees escaped infestation are unknown. These trees were the first to be sampled although C. gemma extracted from other trees within the area was at the pupal stage, thus it is unlikely that the parasite was overlooked in the samples. Tree 8 had an equally low population density for P. ilicis but C. gemma was extracted from within the mines.

Bird attack was an obvious mortality factor since the overall sample site was near to the main nesting area for several species of birds, within the University field station. Owen (1975) in his investigation of the efficiency of blue-tits, Parus caeruleus preying on P. ilicis found that attack by birds was influenced by the number of prickles per leaf. Two trees were examined of different phenotypes, Holly 1 averaged 9.4 prickles per leaf, while Holly 2 averaged 1.4 prickles per leaf. He found that Tree 1 was twice as infested as Tree 2, but blue-tits were twice as successful in extracting larvae from the leaves of the latter. The results suggest that the birds were more able to remove larvae from the less prickly phenotype and that prickles to some extent prevented efficient exploitation of P. ilicis. The blue-tits however had the same effect overall on the trees, successfully finding 16% of larvae on the leaves of each tree, although there were twice as many larvae on Tree 1 as Tree 2. He concluded that predation was density-dependant but the reverse of the normal situation where birds take proportionately more of the prey when it is at high levels compared with low density, and suggested that the tree with more prickly leaves provided a safer enviroment for P. ilicis larvae. During this study variation in the number of leaf prickles and the degree of shinyness of leaves was observed

between some trees, especially at higher elevations, where the number of prickles per leaf tended to be reduced. At these heights infestation by P. ilicis was low. It is possible that few eggs are laid in these leaves because protection from predators is poor. It is equally likely that since the number of leaves at the top of the trees tend to be reduced the amount of useful host material is reduced, thus P. ilicis populations are generally lower compared with other areas. Hence reinfection by previous seasons' adults will be low at these elevations simply because less adults are available. Fig. 39 illustrates the increase in bird attack with total mine number per sample. The straight line graph is typical of a relationship expected from a random search by the birds without searching images being formed. However Tinbergen (1960), suggested that prey species are overlooked by searching birds when available in low numbers, thus the proportion taken will be less than expected on a random basis. As the number of prey increases, encounters with them also increases, and the birds would learn to consentrate on characteristic clues from the prey i.e. a specific searching image is developed. Random search is uncommon since most predators will tend to spend more time in areas where the prey are more numerous. Graph 40 indicates the increase in % bird attack with increasing total mine number. The curve appears to level out at total numbers of 800 mines per sample. Fig. 41 illustrates the increase in mine attack against the total number of viable mines in the sample, again a curved relationship is observed with the curve levelling off above 700 mines per sample. These results suggest that as the total viable mine population increases, the birds learn to search more effectively for the prey, thus increasing the proportion of prey taken as their density increases. The response may level off because of the combined effects of satiation and handling time. Such a response is typical of most vertebrate predators. When the prey density is low the predator will concentrate on other prey or seek areas where the prey density is higher, and this would account for the variation in bird attack between trees.

Chrysocharis syma was the most important pupal parasite and was recorded from all trees (4.3). Trees most infested by the parasite were Trees 4 and 6, where values of 55.56% and 56.86% were obtained. Lowest values for Trees 1, 7 and 8 were found with corresponding values of 4.03, 11.76, and 5.8%. Sphegigaster flavicornis was sparse for all trees, highest levels being recorded from Trees 4,5,6,9,10 and 11. C. gemma accounted for 48.53% of parasites recorded, C. syma 34.01%, S. flavicornis 15.35%, and unidentified parasites accounted for 2.14%. Of pupal parasites only, C. syma accounted for 66.07% and Sphegigaster flavicornis 29.82% whilst unidentified parasites accounted for 4.11%.

During 1937 - 38 Cameron (1939) working in Buckinghamshire, Surrey, New Forest in Hampshire and Forest of Dean in Gloustershire, found the order of abundance of parasites to be a) Chrysocharis gemma, b) Sphegigaster flavicornis, followed very closely and for some areas was superseded by Chrysocharis syma.

Fig 39.

Total number of mines plotted against total number of Bird-Pecks.

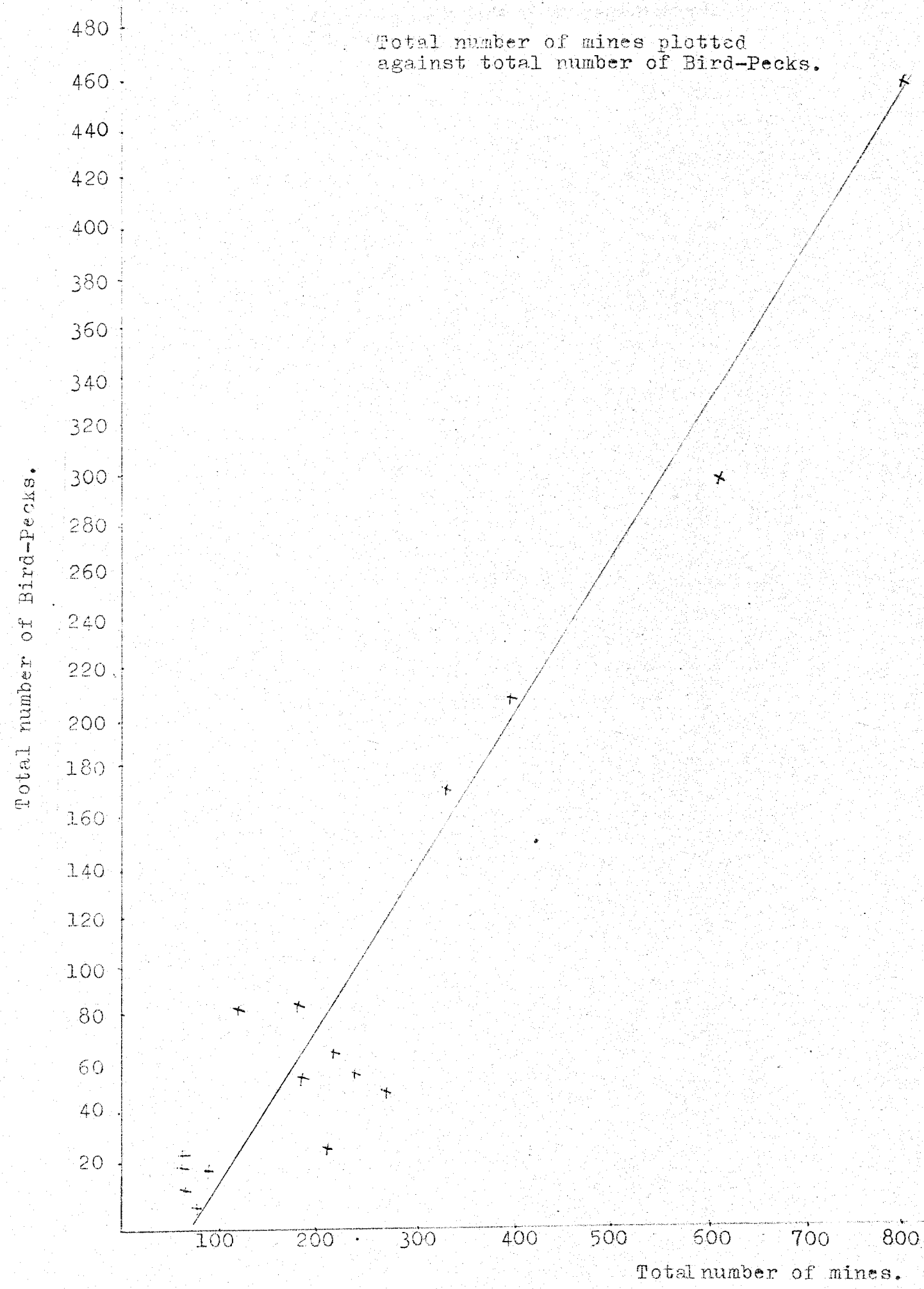


Fig.40

Percentage attack by Bird-Pecks plotted against total mine number.

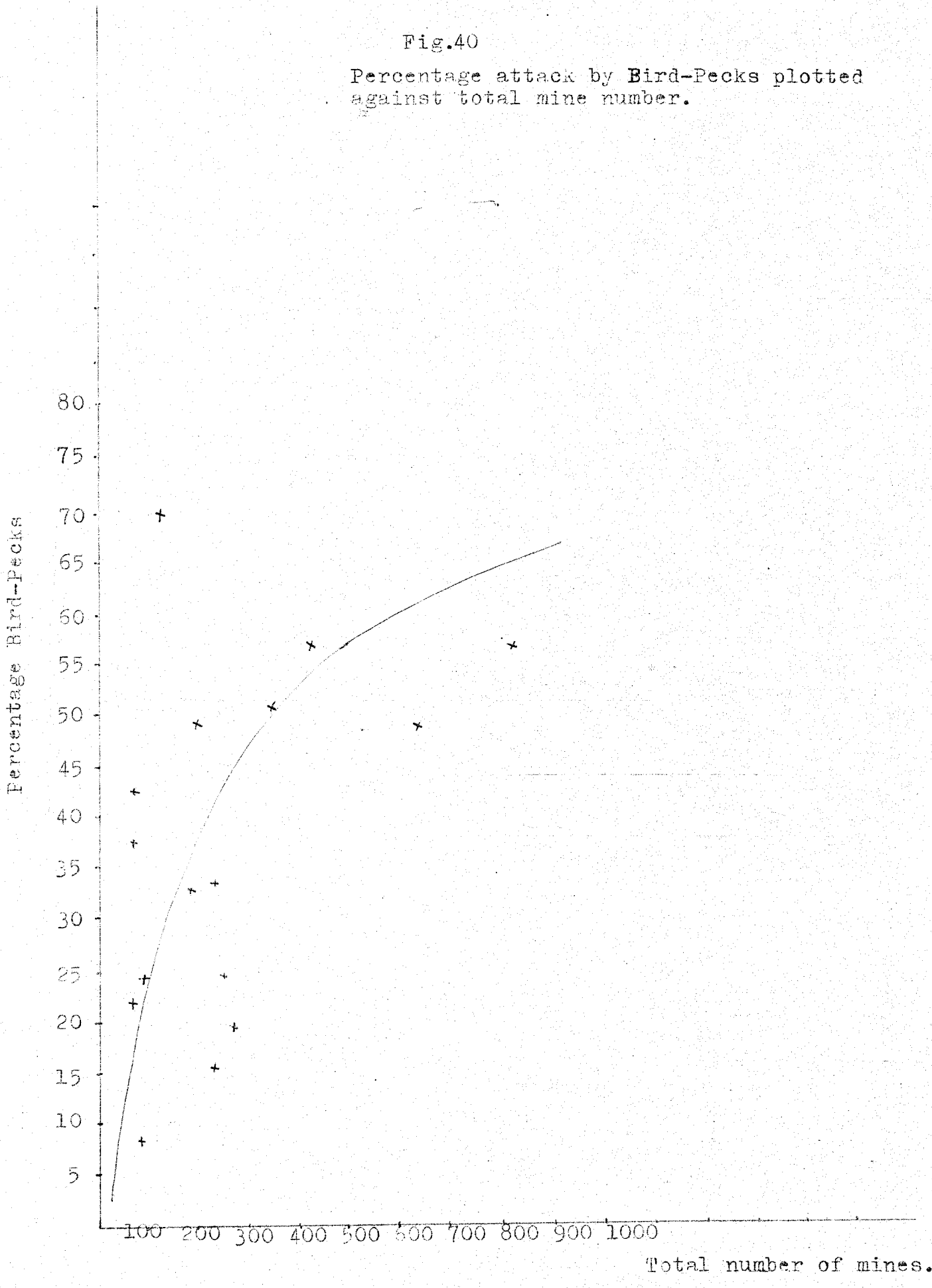
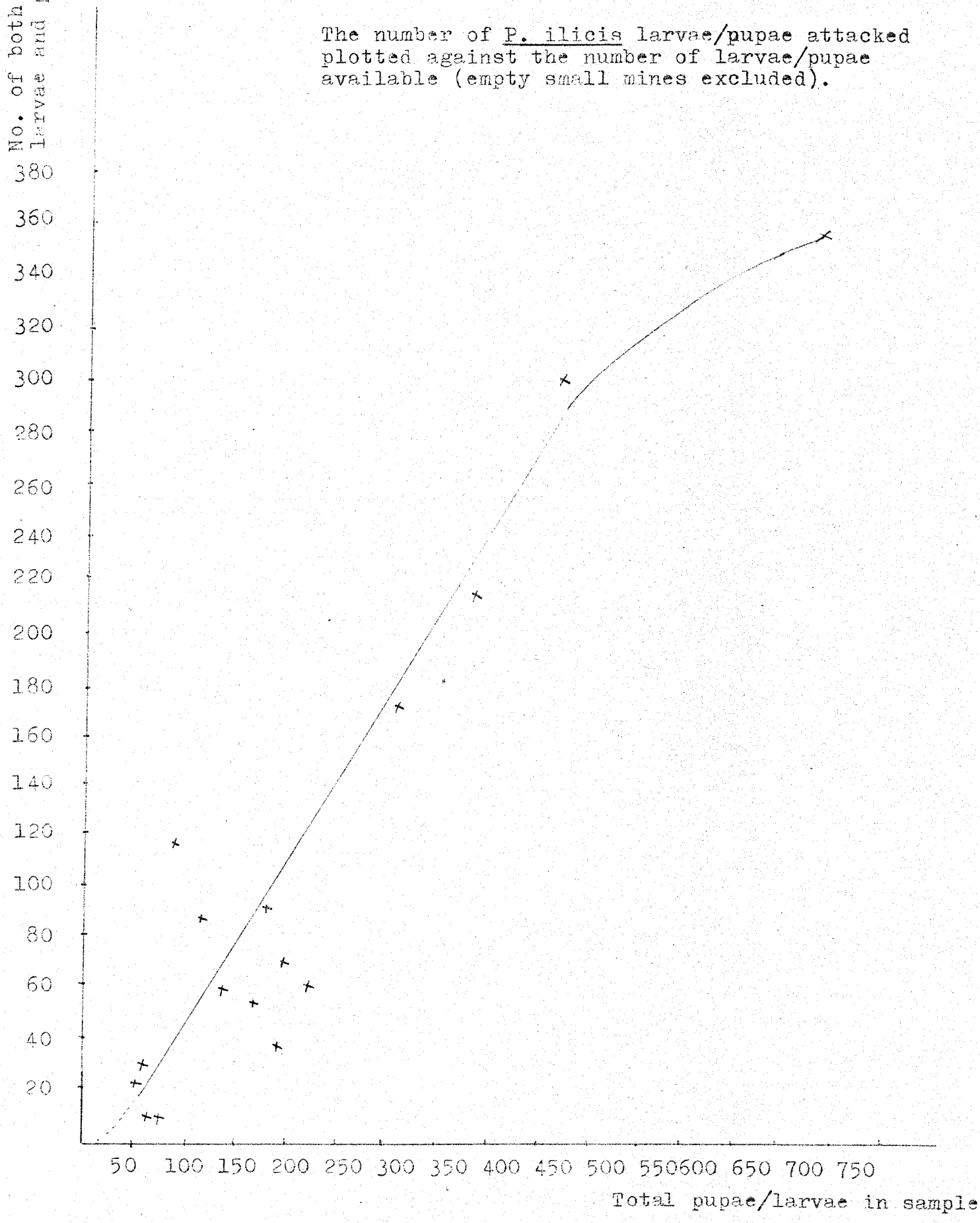


Fig. 41.

The number of P. ilicis larvae/pupae attacked plotted against the number of larvae/pupae available (empty small mines excluded).



He observed that 30 - 40% of fly larvae were found to be attacked by C. gemma, and is in accordance with results obtained in this investigation. C. syma extracted from pupae at Burnham Grove Bucks, were found to range from 0 - 13.8% and is considerably lower than estimates observed for the Durham area. Sphegigaster flavicornis infestation rates varied between 0 - 16.6% and is in accordance with results obtained to date. Obviously the extent of parasite infestation depends on the number of host pupae available but results obtained were conclusive and C. syma was found to be the domimant pupal parasite.

Thus summarizing for the entire sample area, for 100 eggs initially laid :-

1. 25.01 suffer from egg mortality.
2. 32.03 are attacked by feeding birds.
3. 9.91 suffer from larval parasite attack.
4. 3.27 are unhealthy and shrivelled possible due to bacterial infection, or as a result of competition for food where these larvae were extracted from leaves having more than one lime per leaf.
5. 9.57 suffered from early mortality factors.
6. 10.51 were attacked by pupal parasites.
7. 9.85 eggs survived to produce viable healthy pupae which were assumed to emerge successfully.

Thus for 100 eggs originally laid, the overall survival is very low with only 9.85 eggs surviving all mortality factors to the pupal stage.

For the new seasons leaves, of the trees examined up to 17.7.78 when the investigation ended, Tree 15 appeared to have the maximum number of P. ilicis eggs. Trees least affected were Trees 5 and 16 (Table 33). From Table 28, trees most infected were Trees 15, 5, and6. Least affected were Trees 1 and 2, thus for maximum infestation it would appear that trends are constant. It would be expected that trees with nigh levels of infestation would be those where the pupal survival is greatest,(Table34) i.e. Trees 1, 7, and8. Although to date this was not the case no valid conclusions can be drawn until the end of the egg laying period.

Summary of the number of eggs for 1978 season laid per leaf. up to 17th July, 1978.

Table ..33.:

Tree No.	Eggs per 100 leaves	Frequency for Egg No. 1.	2.	3.	4.
1.	31	26	5	-	-
2.	46	26	4	4	-
3.	41	29	9	4	-
4.	40	28	6	-	-
5.	12	10	1	-	-
6.	65	34	10	1	2
7.	39	21	8	-	-
8.	20	16	2	-	-
9.	54	34	10	-	-
10.	Not sampled	-	-	-	-
11.	40	18	8	2	-
12.	48	17	8	5	-
13.	20	16	2	-	-
14.	37	24	2	3	-
15.	74	39	13	3	-
16.	13	7	3	-	-

Table ..34..

Number of pupae avoiding parasitism expressed as % of total number of pupae recorded per tree.

Tree No.	% Healthy Pupae
1	90.40
2	64.00
3	61.96
4	18.56
5	51.57
6	17.65
7	76.50
8	88.24
9	35.50
10	23.78
11	31.25
12	37.00
13	48.54
14	41.43
15	54.00
16	51.80

6. Conclusions.

A significant difference in infestation between the sixteen sampled trees was found at 5% level of significance, but this was insignificant at 1% level. The overall level of infestation was independant of both the height and diameter of the trees. Total mine number and egg density varied significantly between trees. It was assumed that spatial isolation would influence the level of infestation, since trees close together are more liable to reinfection by P.ilicis. from neighbouring trees than isolated trees. Although lowest values were observed for Trees1 and 2, and these were also the most isolated, little diffegence was found in population density between moderately spaced trees and those within a restricted area. Both aspect and height were observed to influence egg/mine density. Highest population density was recorded generally from the East side. Maximum egg numbers were observed between 100-150cm height interval, population density falling considerably at heights above 300cm. Reasons for the differences in egg/mine density within and between trees are many(section 5), but the most obvious factor influencing the number of eggs laid is the availability of susceptible host material. Cuticle thickness was not found to influence whether a leaf was mined or not when comparisons between cuticle thickness for mined and unmined leaves were compared.No significant difference in cuticle thickness between trees was also observed. Cuticle size however is important since adult P.ilicis can only mine young holly leaves, and as the leaves develop they become resistant to attack.

Maximum number of eggs per leaf observed was 5, from which only 2 was observed to survive to the larval stage. Viability was reduced with increased egg density, and this was thought to reduce competion between individuals for both food and space hence increase the chance of survival of any one indiviual larva. Several theories are postulated and are outlined in section 5. Egg density was found to influence the viability of eggs within the leaf but the relationship between egg mortalit and egg density was density-independant.

Mortality of larvae within the mines was caused by bird-attack, attack by the larval parasite C. gemma, and by an unknown mortality factor influencingsurvival in the early stages of development. The major source of mortality was by bird-feeding followed by parasite attack. The pupal parasites recorded during the investigation were C.syma and S.flavicornis. C.syma was the most common pupal parasite, but when all parasite species were considered,C. gemma was the commonest.

Egg mortality was greatest for Tree8,and least for Tree 16, while attack by feeding birds was most severe for Tree2 and least severe for Tree 16. Attack from larval parasites was greatest for Tree 16,the former having least effect upon Tree11. Pupal parasites had greatest effect upon Tree14 and least effect upon Tree8. Tree10 ,although it was not recorded as having a nigh value for the number of pupae avoiding parasitism, the assumed successful emergences are greatest.

For the new seasons growth more samples throughout the egg laying period are required before any valid conclusions can be drawn.

7. Appendix 1.

Rawlins Fluid (universal fixative)
100 cm 50% alcohol.
6.5 cm Formalin.
2.5 cm Glacial acetic acid.

Appendix 2.

Difference in infestation with aspect.

Calculated x^2.	Tabulated x^2 (5%sig).	Significance.	Aspect.
6.40	7.81	Not sig.(mines)	Tree 1.
13.11	7.81	Sig (eggs)	
15.08	7.81	" (mines)	Tree 2.
30.35	7.81	" (eggs)	
17.03	7.81	" (mines)	Tree 3.
28.70	7.81	" (eggs)	
18.17	7.81	" (mines)	Tree 4.
31.43	7.81	" (eggs)	
7.19	7.81	Not sig.(mines)	Tree 5.
15.22	7.81	Sig. (eggs)	
1.26	7.81	Not sig.(mines)	Tree 9.
1.31	7.81	Not sig.(eggs)	
21.95	11.07	Sig. (mines)	Tree 1.(height
35.95	11.07	" (eggs)	

Appendix 3.

x^2 for sig.diff. with aspect and height.

15.95	9.49	Sig. (mines)	Tree 1.
21.52	11.07	" (mines)	
17.65	9.49	" (mines)	
30.43	11.07	" (mines)	
47.34	15.51	Sig. (mines)	Tree 3.
68.95	15.51	" (eggs)	
2.03		Not sig.(mines)	Tree 6.
2.13		" (eggs)	
10.43	5.99	for initial sample	Tree 10height
53.37	14.07	" (mines)	"
80.45	14.07	" (eggs)	"

Appendix 4.

Differences in egg density between trees.

Calculated x^2	Tabulated x^2	significance
50.24	25.0	sig.

Difference in mine density between trees.

33.85	25.0	sig.

Difference in C.gemma attack between trees.

32.37	25.0	sig.

Difference in C.syma attack between trees.

111.28	25.0	sig .

Difference in the no. of non-parasitised pupae between trees.

150 83	25.0	sig.

Difference in empty mine no. between trees.

17.24	25.0	not sig.

Difference in the larvae-pupae survival between trees.

25.43	25.0	not sig.

Difference in the no. of bird-pecks between trees.

22.85	25.0	not sig.

Difference in the % egg mortality between trees.

23.15	25.0	not sig.

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